This month’s issue features A2 milk, casein proteins, dairy and genetics, and breastfeeding and allergic disease.

Do You Have Any Dairy Desserts Tailored to My Genotype?

- Each person on average has about three million genetic variations compared with another person.
- Cow’s milk has many components that when consumed by humans may contribute to protection against various diseases, but sometimes these responses are modified by specific genetic variations in individuals.
- In the future, diets may be personalized to the unique genetics of individuals to help optimize their health management, but there is still a lot to learn.

Senior citizens celebrating a centennial birthday are often asked the secret of their good health and longevity. The crowded room is silent in anticipation as the young listen intently to their prophet’s every word. The reply sends seismic tremors through the modern health care system – “I’ve always been big, likely because I ate everything, mostly the foods they say you shouldn’t and no greens, I smoked like a chimney, drank a bottle of whiskey every day, and importantly, never exercised.” This individual, of course, is not representative of the average person. Health scientists and doctors regularly report that such lifestyle choices are strongly associated with chronic ill health and premature death for most people.

Thus, for this elderly person, the often-used phrase “you are what you eat” should be replaced by “you are healthy, despite what you ate.”

Why is this individual different? Within large populations there are a few people that just happen to be on the lucky side of the bouncing ball of life. Part of this luck is that their unique genetics interacts favorably with local environmental influences and dietary choices, perhaps not the whiskey, and these interactions alter their susceptibility to chronic lifestyle-related diseases. Of course, the opposite is also true as the genetic lottery of life produces winners and losers.

**Nutrigenetics and You**

Mapping how ingested foods interact with the unique genetic makeup of an individual to affect their health is a rapidly expanding area of science called nutrigenetics. The aim of nutrigenetics is to provide people with personalized nutritional advice based on their genetic makeup to allow better management of their health. Recently, Kevin Comerford and Gonca Pasin* published an insightful and timely review summarizing about fifteen years of scientific investigations in this new research area [1]. Their review, published in the journal *Nutrients*, was focussed on whether the specific genetic makeup of individuals interacted with ingested dairy products to modify the risks of metabolic diseases, heart disease, bone health and some cancers [1]. The reviewers are associated with the California Dairy Research Foundation. This is a monumental review as Comerford and Pasin distilled information from about thirty large-scale investigations that in total used health, diet and genetic information from about half a million people. They deftly extracted simple biological conclusions from the complex statistical analyses underpinning each investigation as well as consensus views from multiple related investigations. So, what did they conclude? Overall, these studies indicate that dairy products are very good for the health of people, although the genetic makeup of some individuals can influence these benefits in special circumstances [1].

**Genetic Influences are Everywhere in Biology**

Genetics is a foundation pillar of biology. It links the generations of all life forms by transmitting a code defining form and function. Nowhere is this more apparent than in domesticated animals. The different milk production characteristics of cattle breeds and the amazing visible variety of dog and cat breeds
exemplify the awesome power of genetic variation coupled to selective breeding. In the human population, only about 0.1% of the entire genetic makeup is variable. These three million genetic variations generate massive individual variety; just look at the local crowd [2, 3]. Many traits with a strong genetic basis, such as human height, are influenced by the actions of hundreds of genetic variants, each providing only a small contribution to the trait [4]. This is a complex or polygenic (many gene) trait that geneticists still struggle to fully understand. Most of what is known about genetics and its relationship to form and function is derived from single genetic variations (mutations) that cause large and easily seen changes in individuals. For example, many individuals in 19th Century European royal families carried a mutation on the X-chromosome causing hemophilia, a severe blood clotting disease resulting in abnormal bleeding. A female inheriting two copies of the mutation or a male inheriting one copy will get the disease, i.e. the mutation determines the disease [5].

Most mutations are loss of gene function mutations that cause adverse effects. It’s like damaging a random part in a car engine. Most times the engine does not work as well or sometimes not at all. Very rarely, mutations cause a gain of gene function that results in an advantage to the individual carrying the mutation and sometimes secondary effects as well. Randomly playing with switches on the car dashboard occasionally turns on a car’s air conditioner, an important gain of function on a hot day, but it also increases fuel consumption. Genetics often represents the potential for a biological effect but only in a specific environmental circumstance. During cold weather, the air-conditioner is turned off and the fuel consumption is normal. The divide between nature and nurture starts to get blurred.

Comerford and Pasin highlight the lactase persistence (LP) mutation as an example of a gain of function mutation as it allows some adult human populations to drink milk without suffering the effects of lactose intolerance [1, 6, 7]. In mammals, lactose in milk is broken down in the gut of the young into easily metabolized glucose and galactose. The gut protein that performs this task is lactase, which is abundant in the suckling young, but after some time it is usually no longer produced and this leads to weaning. Uniquely in humans, different mutations located at or near the gene coding for lactase arose in the “recent” past (one mutation arose about 10,000 years ago) and led to the persistence of lactase production into adulthood in three separate populations. The mutations gave these ancient human populations a big calorie advantage through the ability of the adults to consume cow’s milk containing energy-rich lactose and other beneficial components. The nutritional advantage gained by these populations greatly enhanced their survival chances, allowing population expansion as the mutations were efficiently passed onto subsequent generations. This is an example of evolution in action. Today, these inherited LP mutations are prevalent in populations characterized by Northern European ancestry and in smaller groups with African and Middle Eastern ancestries.

Times change, and so does the nutritional environment. Modern human populations carrying the LP mutation often live in a world of food abundance and no longer need the mutation’s calorific advantage. Comerford and Pasin’s review was specifically focussed on investigations by scientists that were interested in whether the LP mutation, interacting with a dairy diet, modified the usual line-up of recalcitrant risk factors underpinning common modern-day diseases. The reviewers noted that several investigations using small human populations demonstrated that the most prevalent LP mutation was correlated with higher dairy intake, as expected, and a higher body mass index (BMI) [8-11]. BMI is an established risk factor of several diseases including heart disease and type II diabetes. However, investigators involved in a much larger study of 98,000 people found no evidence that the LP mutation and dairy intake were associated with BMI [12]. These apparently contradictory conclusions were attributed to additional but as yet unknown genetic, dietary and lifestyle factors that were different in these investigations.

Using another set of investigations, Comerford and Pasin concluded that there is strong and independently confirmed evidence that adult dairy intake showed neutral or protective associations with risk factors for cardiovascular disease [13] (and citations numbered 37-41 in Comerford and Pasin’s review). This is good news for dairy consumers. One study however, showed that the LP mutation in combination with a high dairy intake increases the likelihood of metabolic disorders that are risk factors for coronary heart disease but only in women [14]. A related study could not confirm this association [15]. Moreover, the reviewers reported on results from four very large investigations using data from 20,000, 197,000, 98,000 and 102,750 participants, which all concluded there is no association between the LP mutation, dairy intake and risk factors for heart disease [12, 16-18].

The reviewers summarized the extensive evidence that milk consumption strengthens bones and is
associated with long-term bone health (citations 56-61 in Comerford and Pasin’s review). These investigators demonstrated that multiple components in milk, such as total protein and calcium, support bone health. The role of milk lactose in these beneficial associations is unclear. Lactose intolerance (i.e. lack of the LP mutation) is associated with increased risk of osteoporosis and bone fractures [19, 20]. However, lactose intolerance is also associated with decreased dairy consumption [8-11]. Therefore, the reviewers suggested that the decreased consumption of milk protein and calcium, and possibly not the decreased ability to metabolize lactose, may be responsible for the increased risk of osteoporosis and bone fractures in lactose-intolerant populations. The reviewers also highlighted two additional investigations concluding that there was no effect of lactose tolerance (an indirect measure of the presence of the LP mutation) or lactose intolerance on bone health associated with specific groups of post-menopausal women [18, 21]. Overall, Comerford and Pasin concluded that multiple genetic, dietary, and environmental factors influence the risk of osteoporosis and bone fracture, thereby confounding some investigations that narrowly focussed on a single genetic mutation and milk intake in these populations.

A similar conclusion was reached by the reviewers when they examined multiple investigations into the role the LP mutation in the risk of various cancers. The reviewers noted substantial evidence implicating dairy intake with protection against colorectal cancer, but most of these investigations did not examine the effects of the LP mutation or any other genetic variants [1].

Effects of Non-Lactase Persistence Genetic Variants

Comerford and Pasin also summarized nine additional investigations where the health effects of genetic variants in a variety of other genes were modified by dairy diets. The genetic variants investigated were present in genes important to fat metabolism, the biological activities of vitamin D, and the functions of several hormones [1]. The reviewers highlighted many interesting interactions; however, overall, they concluded that these nutrigenetic investigations showed “mixed results,” and implied that multiple unknown genetic and dietary factors were influencing the risks of disease.

Implications

In the future, each individual will know their genetic makeup and how it conspires with dietary components to influence their long-term health. Comerford and Pasin summarized the first tentative steps toward this ambitious goal by highlighting interactions between a few genetic variants, a single dietary component—milk—and the risk of some common diseases. Undoubtedly, their conclusion is that these analyses are complex and involve many known and hidden interacting factors. Perhaps that is the point. Individual differences in disease susceptibility and type may reflect the sum of many small contributions derived from an array of genetic and environmental factors. There is obviously still much to do. Perhaps one day in the future, it may be routine to ask the waiter “Do you have any dairy desserts tailored to my genotype?”

*This article is dedicated to the memory of one of the review authors, Gonca Pasin, a champion of the dairy industry and a very nice person.


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Relationship Between Breastfeeding and Allergies: It’s Complicated

- The first six months of life offer a window of opportunity for preventing allergies, and as a result there’s been a lot of interest in the effects of human milk on allergies.
- A recent review finds that although modulating human milk composition may have the potential to prevent allergies in early life, studies have so far shown conflicting evidence about the protective role of breastfeeding on allergies.
- Breastfeeding is associated with a reduced risk of eczema in the short term but not at 6-7 years of age.
- The conflicting data may be due to variations in study methodologies and outcome measures, as well as heterogeneity in human milk composition.
- Large, standardized studies that account for several constituents of human milk at the same time could help uncover its potential protective effects on allergies.

The past few decades have seen a steady rise in the worldwide prevalence of allergic diseases, which has spurred research aimed at figuring out ways to prevent allergies [1]. The first six months of life are thought to offer a window of opportunity for preventing allergies. “Nowadays, most researchers and clinicians are trying to aim at this window of opportunity,” says Professor Daniel Munblit of Imperial College London, Sechenov University, and inVIVO Planetary Health.

One area that researchers are focusing on is the effect of breastfeeding on allergy risk [2-4]. “With regards to breast milk, since ideally speaking this should be the only food for the baby in this first six months of life, it seems reasonable to aim at human milk composition to improve it or somehow modify it to reduce the risk of allergic disease development,” notes Munblit.

Human milk is known to be very complex, with variable levels of immune-active molecules, proteins, polyunsaturated fatty acids, oligosaccharides, metabolites, vitamins, and other nutrients and microbial content, and many of these components interact in ways that can affect immunity [5-8]. Breastfeeding is known to help alter the infant gut microbiome and subsequent immune development [8,9]. “It looks like breast milk potentially can have an impact on immunity,” says Munblit. “Overall, I think that breastfeeding is beneficial in a number of ways.”

Interventions that could modify human milk to influence infant immune responses could potentially help reduce the risk of allergies in early life. However, a recent review by Munblit and his colleagues reports that studies have so far shown conflicting evidence about the protective role of breastfeeding on the risk of allergies [10]. The review suggests that the conflicting data may be explained by variations in study methodologies and outcome measures, as well as heterogeneity in human milk composition. Researchers will likely need to conduct more standardized studies that look at human milk as a whole, rather than as individual components, to decipher any potential protective effects of human milk.
on allergies.

Munblit was a masters student at Imperial College London when he first started looking into the association between human milk and allergic diseases, and he continued to work on this topic during and after his PhD. “My main interest is in the immunological components of human milk,” he says.

Several components of human milk are known to influence immunity and could play a role in reducing the risk of allergies. “Talking about breast milk composition, there are some immunological molecules like TGF-beta and soluble CD14 that can potentially make a difference,” notes Munblit. Milk lipids, particularly polyunsaturated fatty acids such as the omega-3 fatty acids, including docosahexaenoic acid, have also been shown to have anti-inflammatory effects in chronic inflammatory diseases such as asthma [11].

Other components of human milk, such as sugars called oligosaccharides, have been associated with a direct protection against infections and may help reduce the incidence of allergic diseases in breastfed infants later in life [12,13]. Dietary supplementation with specific oligosaccharides has also been shown to reduce the risk of developing allergies in infants [14,15].

Human milk is also known to shape the infant’s gut microbiome, which can influence the risk of non-communicable diseases in later life, including allergies [16-18]. The gut microbiome of children with allergies also differs in composition and diversity from those without allergies [19].

However, despite its known beneficial effects on infant immunity, there is conflicting evidence about the protective role of breastfeeding in relation to the development of allergies. Many studies have tested breastfeeding associations with the onset of allergic sensitization, eczema, and asthma, and the results have been inconsistent.

“With regards to allergic diseases, the results are quite mixed,” says Munblit. “For example, we can see that breastfeeding is associated with a reduced risk of eczema in the short term, but then when you look long term, by six or seven years of age, this effect disappears,” he notes [20,21]. “I don’t know why it happens, maybe there are some additional confounding factors that we don’t take into account,” says Munblit. “There’s still a lot unknown, despite breastfeeding research being embraced for decades, maybe even centuries.”

A systematic review and meta-analysis of eczema research suggests that children under the age of two who were exclusively breastfed for more than 3–4 months were at lower risk of eczema development, but this protective effect was no longer evident after the age of two [20]. Another large observational study assessing more than 200,000 children worldwide reported that breastfeeding offered some protection against severe eczema but failed to find evidence of a protective effect on eczema development at six to seven years of age [21].

Studies assessing the association between breastfeeding and food allergy have also had conflicting results. Some studies reported a reduced risk of food allergy development while others suggested a greater risk after breastfeeding [22-25].

Multiple studies have found associations between breastfeeding and lowered asthma prevalence in children; breastfeeding also appears to reduce the number of respiratory tract infections and risk of respiratory failure in infants [20,26-29]. However, these studies are all quite heterogeneous, with very different study designs and outcome definitions, making it difficult to draw unequivocal conclusions from them.

Breastfeeding is also associated with an increase in the size of the thymus, an essential organ for generation of T cell immunity and tolerance. Thymus size at four months of age in exclusively breast-fed infants was more than double the size in formula-fed infants, and this effect persisted at least until 10 months of age [30]. Breastfeeding between eight and 10 months also correlated with increased thymus size [31]. However, it is still unclear how this breastfeeding-related increase in thymus size occurs and how it affects immunity.

Another complication when investigating the effects of breastfeeding on allergy is that most current studies are observational, which means they can provide information about the association between the two factors but are unable to provide concrete evidence of any causal relationships. Randomized trials—where one group of infants would be randomly assigned to be breastfed while another group isn’t—would help establish causal relationships, but such studies would be unethical.

“It’s virtually impossible to randomize breastfeeding,” says Munblit. “You can’t just tell a mother, ’I think you shouldn’t breastfeed,’” he says. The only randomized trial so far was conducted in Belarus, a country with very low breastfeeding rates, where mothers were randomized to a breastfeeding promotion group or continued standard practice [32]. The researchers found a reduced risk of early eczema with breastfeeding but no long-term protection against eczema, allergic rhinitis, and asthma at 6.5 years of age.
One of the reasons behind all these conflicting results may be differences in methodology, because if you look the data is quite heterogeneous,” says Munblit. “There are really two very distinct types of studies: breastfeeding studies that assess breastfeeding association with regard to health outcomes, and other studies that assess human milk composition with regard to health outcomes,” he notes. “But there are no studies looking at both simultaneously,” says Munblit. “Also, for a long time there was a lot of heterogeneity in terms of breastfeeding definitions, and many people defined exclusive breastfeeding not as per the WHO definition,” he says. “Many systematic reviews and meta-analyses had to deal with many studies that were undertaken with very mixed definitions,” notes Munblit.

Human milk is also quite heterogeneous, which could explain differences between different studies [33]. “Current research lacks studies looking at breast milk composition as a whole,” says Munblit. “Those conflicting results that we see in the studies may be linked to breast milk composition, because composition varies from one woman to another, but it is not taken into account in large prospective breastfeeding studies.”

“What we need are really large sample size studies assessing multiple immunological factors, the microbiome, etc.,” says Munblit. “If we just look at the proteins in breast milk, there are more than 300, and in most of the studies only 10 or 15 are assessed, which means we are just assessing a very limited amount of immunological character,” he says. “So there’s a lot of work to be done.”

To account for the heterogeneity in human milk composition, future studies could attempt to characterize lactating women according to the constituents of their milk, forming so-called “lactotypes.” They could then examine whether different lactotypes are associated with specific immunological outcomes.

“What we hope to find are distinct lactotypes, maybe for women living in a particular geographic location, or maybe a particular lactotype, which is associated with the later development of a particular non-communicable disease,” says Munblit. “This is very preliminary, just a hypothesis, and something we would like to look at in the future,” he says.

Researchers are also increasingly beginning to consider the effects of food proteins, such as ovalbumin, in human milk, on allergies. “Maybe this is also a piece of the puzzle, because we know that food proteins are excreted into breast milk, and the degree of this excretion really varies from one mother to another,” says Munblit. “This is a really interesting topic, and we will most definitely see more data on the subject within the next year or two,” he suggests.

Researchers are interested in finding out whether interventions to modify human milk can influence allergy development in early infancy. Maternal lifestyle, including dietary habits and physical activity, can have a significant influence on the biologically active components of human milk [34-37]. Modifying one or more of these factors could potentially affect infant immunity. Probiotic administration, for example, may offer some protection against eczema [38]. But it’s still early days, and it’s unclear how well such interventions will work. “The basis of this research is quite controversial,” notes Munblit. “Some colleagues of mine believe that it is possible to modify breast milk composition, but others don’t believe that it would be possible to make this change.”

“Probiotics and prebiotics, fish oil, and fresh fish consumption are the interventions usually used for this purpose,” says Munblit. “Looking at some studies, fresh fish consumption is linked to changes in omega-3 and omega-6 fatty acid ratio in breast milk, and since it is assumed that polyunsaturated fatty acids are responsible in part for non-communicable disease risk reduction, including allergy, that brings some hope that we may influence breast milk composition by means of maternal diet changes,” he says. “We could then subsequently influence health outcomes, including allergic diseases.”


Milk Casein Proteins: Ancient, Diverse, and Essential

- Casein proteins are unique to milk and provide infant mammals with essential amino acids, and also bind calcium and phosphorus required for skeletal growth.
- Milk contains several types of casein proteins, which are highly diverse both across and within mammal species.
- Interest in cow milk casein composition has peaked due to the arrival of A2 milk in the dairy case.
- A2 is one of 13 different beta-casein proteins, each of which contributes to milk’s high protein and calcium content.

Grab your nearest carton of milk. Find the nutrition label. Under total fat, you’ll likely find information about how much of that fat is saturated, unsaturated, and even trans fatty acids. Under carbohydrates, you’ll learn how much fiber and sugar your milk contains. But there is just one row of information when it comes to protein, giving the false impression that milk protein is not nearly as complex as milk fat or sugar. However, cow milk is made up of two different types of proteins, whey and casein, the majority of which are caseins. There are four different subtypes of casein proteins, and for each of the four subtypes, there are dozens of different genetic variants. How’s that for complex?

Until recently, just which types of casein proteins were in any given carton of cow milk was never of much concern. But that may be changing with the arrival of A2 milk in dairy cases across the U.S., and claims that some casein proteins are healthier than others. Simply knowing how much protein is in a glass of milk may no longer be sufficient; it’s time to get complex, move beyond the nutrition label, and understand the diversity of caseins in your glass of milk.

Meet the Caseins

Milk of all mammals, from the egg-laying platypus to the social-networking human, contains a mix of whey and casein proteins. In humans, the concentration of the two is almost evenly split, with 60% whey and 40% casein. In cows, caseins dominate, comprising nearly 80% of the milk proteins. These differences in proportions relate to different developmental needs of human and cow newborns. Whey proteins are easier to digest, and as a result, provide a more rapid source of amino acids. In contrast, the unique structure of casein proteins—called the casein micelle—makes them harder to break apart and requires a longer digestion time.

Both whey and casein proteins provide mammal infants with the amino acids needed for growth and development. But casein micelles provide something else important for growth: calcium and phosphorus. The unique packaging of proteins and minerals is only accomplished by cells in the mammary gland and allows for these nutritious molecules to be suspended in liquid (milk is, after all, mostly water) as if they were soluble (even though, technically, they are not).

Casein micelles contain two types of casein proteins, calcium-sensitive (which includes the three subtypes αs1-, αs2-, and β- caseins) and calcium-insensitive (κ-casein only). The three calcium-sensitive caseins are responsible for binding the calcium and phosphorus, whereas the κ-casein is responsible for stabilizing the structure [1]. When these protein-mineral spheres reach the digestive tract, specific digestive enzymes snip away the κ-casein, turning the once soluble micelle into an insoluble curd. This may sound like a bad thing, but in fact, it is of great benefit to all milk consumers because it keeps you feeling fuller longer, and gradually releases nutrients into the bloodstream. What was once liquid is now a solid, requiring more digestion effort and time.

But the time and effort the infant puts into digesting casein curds are worth it—the micelle structure of caseins allows the concentration of calcium and phosphorus in milk to exceed what would be possible if these minerals were delivered on their own [1]. All mammalian newborns and infants have high calcium and phosphorus requirements, as these minerals are essential for skeletal growth. But the requirements for these minerals are particularly high in the oldest lineages of mammals—monotremes (egg-laying mammals) and marsupials (mammals with a pouch)—which give birth to extremely immature offspring. To say that caseins are responsible for the success of the mammalian lineage is not an overstatement. Casein protein's ability to bind calcium and phosphorous, while simultaneously delivering high-quality protein to the neonate and infant, allowed the earliest mammals to successfully reproduce immature offspring in a variety of environments [2,3].

A Diverse Family Tree

The genes that provide the instructions for assembling calcium-sensitive and calcium-insensitive casein
proteins are unique to mammals and are found in all living mammals [2]. Investigations of the casein protein genetic family tree suggest they are most closely related to genes responsible for mineralization in teeth and bones in vertebrates [2]; apparently caseins have always had an affinity for calcium and phosphorus.

Although all mammal genomes have the instructions to make αs1-, αs2-, β-, and κ-caseins, the language of those instructions (the A, C, T, and Gs of the DNA code) differ greatly across and within species [2–4]. Indeed, in a comparison of milk genes among distantly related mammals (e.g., platypus, opossum, cow, and human), casein protein genes were the most divergent of the milk proteins [4]. Rijnkels [3] reports that differences across species in the amino acid sequences of the αs1- and αs2-caseins are primarily due to shuffling of the coding portions of the genes, known as exons. In contrast, β-casein variation is primarily the result of point mutations, a change in just one letter of the DNA code [3].

Changing the words around in an instruction manual is unlikely to result in making a working final product. In the same way, changing around amino acids in the coding portion of a gene usually changes the protein’s function. Such is the case with sickle cell hemoglobin—one letter change in one amino acid in one of the four protein chains that make up the hemoglobin protein produces sickle-shaped red blood cells, which are functionally inferior to their round hemoglobin ancestors in transporting oxygen throughout the body. How can casein protein genes have rearrangements of coding portions or changes in the amino acid sequence without having major functional changes?

The reason may be related to the shape of casein proteins. Many proteins are coiled up tightly, like little balls of yarns. The amino acids that make up the protein chain are either positively or negatively charged, and thus as they coil up they react with one another and alter the shape of the protein. Casein proteins, however, are described as having a more open (or unfolded) shape, with more space between the different amino acids. Thus, mutations that change the amino acids in an open protein are less likely to change the shape, and thus the function, of the protein. As a result, casein proteins can handle frequent mutations better than other types of proteins. They are highly divergent because they can be; caseins are not under the same constraints as other proteins [3].

**Much Ado about A2**

Most consumers may be aware that their milk contains whey and casein proteins, but they could probably not tell you a specific type of cow milk casein protein. That may be about to change for U.S. consumers, however, as A2 milk is becoming more prevalent in local grocery stores. A2 refers to a genetic variant of cow milk β-casein, and it is believed to be the ancestral type of β-casein. A point mutation (a C was changed to an A) resulted in the A1 gene variant over 8000 years ago [5]. It is believed this mutation occurred in European herds, as cows of European descent produce both the A1 and A2 β-casein proteins, but cows native to Asia and Africa have only the A2 allele [5].

A2 milk contains only A2 β-casein proteins, whereas regular (or conventional) milk contains some A1 β-casein, where some is the operative word. Most milk found in the U.S., Canada, and Europe actually contains a combination of A1 and A2 β-casein proteins. The genes for these proteins are considered co-dominant, meaning that A1/A2 cows produce both protein types in milk. If a herd has mostly A2 genes, the milk they produce could contain only a small amount of A1 milk; a predominantly A1 herd will produce quite different milk. Thus, regular milk could be mostly A2, mostly A1, or somewhere in between.

Talking about only A1 and A2 makes it seem like there are only two types of cow β-casein protein, but there are actually 13 [6]. A2 and A1 are genetic variants but are also used to represent "types" of β-casein proteins. This makes it easier to talk about cow milk proteins but masks the diversity of caseins across and within breeds. The eight β-casein proteins grouped under A2 share the same amino acid at position 67 (proline), whereas the five grouped under A1 have histidine at position 67. This difference in amino acid is relevant to the way the proteins are digested. Digestive enzymes cut the A1 β-casein types at position 67, producing a seven amino acid-long peptide called β-casomorphin 7 (BCM-7). Because proline forms a strong bond with its neighboring amino acids, A2 β-casein types stay intact at position 67 and do not produce BCM-7.

The production of a seven amino acid-long chain seems somewhat inconsequential when considering all
the ingredients passed along in milk, but there are claims that BCM-7 may cause digestive issues in some consumers. Currently, there is little scientific support for digestive differences between milks containing some A1 proteins and those containing only A2. On the other hand, both types of β-casein are associated with well-established health benefits derived from their essential amino acids, calcium, and phosphorus.


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What’s in the Dairy Case? A2 Milk

- Milk with the A2 beta-casein protein is marketed as an easier-to-digest alternative for people who suffer gastrointestinal issues from regular milk.
- Digestion of A1 milk releases the bioactive peptide BCM-7, which has been shown to slow the movement of the food through the gastrointestinal tract in several rodent studies.
- Does A2 milk improve digestive issues, such as gas and abdominal pain, relative to regular milk? The evidence is insufficient.
- If consumers tolerate regular milk, there is no scientific reason to switch to A2 milk.

It used to be that the only decision you needed to make at the dairy case was full-fat or low-fat milk. Today, consumers are faced with dozens of alternatives to conventional cow milk, including milks free of lactose and “milks” made from soy beans, nuts, rice, and even peas (more about that in future articles). One of the newest alternatives to hit the shelves is A2 milk. It is marketed as an easier-to-digest version of conventional cow milk, differing by only one amino acid in one protein chain. But does the change in one protein really change the way A2 milk is digested?

Digestive Differences

Milks of all mammals contain a mixture of two major classes of proteins, whey, and casein. Approximately 80% of the protein in cow milk is present as casein, of which there are four subtypes: αs1-, αs2-, κ-, and β- caseins [1-4]. A1 and A2 refer to two of several gene variants on β-casein proteins, which comprise about 30% of all casein proteins [1-5]. Thus, when you drink cow milk, just over a quarter of the protein you are consuming is β-casein [1].

A1 β-casein and A2 β-casein proteins are similar in function but differ in how they are digested. When A1 β-casein proteins reach the small intestine, digestive enzymes break the protein apart and release a seven-amino acid peptide chain called β-casomorphin 7 (BCM-7) [3, 6, 7]. Importantly, this peptide chain is not released during digestion of A2 β-casein proteins. BCM-7 and other milk peptide fragments released during casein protein digestion are biologically active; each peptide interacts with specific tissues, resulting in specific physiological responses. Both in vitro and in vivo animal studies suggest BCM-7 interacts with, and thus influences, specific peptide receptors found in the nervous system, the endocrine system, and the immune system.
There is a link between A1 milk, BCM-7 production, and digestion in animals, but it is not known yet whether these relationships are meaningful in humans. Those that argue that A2 milk is easier for humans to digest need to demonstrate not only that BCM-7 from A1 β-casein influences gut transit time and smooth muscle contractions, but also that these actions result in measurable negative GI issues in humans.

It is important to point out that investigations comparing digestion of A2 milk with regular milk do not assume that A1 proteins cause digestive issues in everyone. Just like some people have an intolerance to gluten protein whereas others can eat an entire baguette with impunity, BCM-7 is hypothesized to influence smooth muscle contractions in the GI tract in only some people (although it is not clear yet why some people may be more sensitive to the actions of BCM-7 than others). Indeed, many argue that people who diagnose themselves as lactose intolerant may actually be reacting to the A1 protein as opposed to milk sugar [1, 2, 8-10]. As a result, most clinical studies comparing digestion in A1 and A2 milk have recruited participants with self-reported lactose intolerance; if A1 issues are present, this is the most likely group of individuals in which to identify them.

To date, there have only been four human studies that follow the gold standard for experimental design (i.e., randomized, double-blinded clinical trials). This type of study minimizes bias on the part of the study participant (they do not know what intervention they are receiving and thus cannot adjust their responses accordingly, they are randomly placed in a study group) and on the part of the researcher (they don’t know who is in each group and thus can’t let their hypothesis influence how they interpret the data). However, despite this highly regarded study design, each of these studies on A1 vs. A2 milk still has an inherent level of bias disclosed in their publications—the research was funded by the A2 Milk Company. Ideally, independent researchers without any financial interests in the outcome of the study would address this hypothesis.

Each of the studies followed a similar crossover design, wherein participants are asked to refrain from eating dairy for a period of time before the study begins to establish baseline values, and then randomly assigned to consume either only A1 or only A2 milk. Then, after another washout period, they consume the other milk type, and results from both intervention periods are compared. Each study differed in the amount of milk and the duration of each intervention, as well as which outcome measures (e.g., stool consistency, stool frequency, abdominal pain) were assessed, which makes direct comparisons difficult. For example, Ho et al. [10] had participants consume 750 ml of each type of milk every day for two weeks, whereas Mei et al. [2] gave each subject 300 ml of each milk at just one time point, and then collected follow up data at 1, 3, and 12 hours after consumption.

Despite these differences, these studies converge on similar conclusions: consumption of A1 milk was associated with a longer gut transit time and softer stools compared with A2 milk consumption and baseline values [2, 3, 9, 10]. This is somewhat suspicious because usually longer gut transit time is associated with harder stool. Regardless, it is important to point out that these conclusions do not necessarily support the hypothesis regarding A2 milk’s easier digestion. Not to get too personal, but slightly softer stool and/or a slightly longer gut transit time need not be indicative of digestive issues. Indeed, in the study by Ho et al. [10], although the participant-assigned scores on the Bristol Stool Scale (that’s right, there is an index for assessing stool consistency!) were higher for A1 compared with A2 milk, mean values for A1 were actually closer to the normal range (which, if you are dying to know, is a score of 4).

The best data for testing the hypothesis regarding ease of digestion are actually subjective data, wherein participants report precisely how they feel after consuming each type of milk. After all, this is what will drive consumer decisions. The largest (and therefore most statistically powerful) study to address this to date comes from Mei et al. [2]. Each of the 600 Han Chinese participants was asked to report the severity of their symptoms at baseline and at set time points after each milk type consumption based on a 9 point scale, where 0 = not at all and 9 = very serious [2]. Using these scores, the study authors created categories to evaluate the differences between A1 and A2 milk for each of the six evaluated symptoms (which included gas, bloating, abdominal pain, and stool frequency). The study reports that there was a greater trend toward slight improvements (a reduction in score of 1–≤3) in GI symptoms when
participants consumed A2 compared with A1 milk [2]. But the way in which the researchers composed their categories to evaluate differences between treatment interventions was extremely problematic. Namely, all of the categories capture only an improvement of symptoms with A2 (a reduction in score from A1 to A2). The category for worsening of symptoms from A1 to A2 (or an increase in score) is actually included within the category of no change in score. Without access to the raw data, readers are left to wonder how much of the 65% of study participants who experienced no difference in abdominal pain or the nearly 70% of participants who experienced no difference in stool frequency may have actually had worsening of symptoms with A2 milk. One would assume that this number is not zero, as the study authors would have been able to omit “worsening of symptoms” from the no difference category altogether. But is the number statistically significant? In order to take their conclusion of slight improvements in symptoms as valid, the research must be more transparent than its current state.

**Is A2 Right for You?**

The science of A2 milk is still in its infancy. Any scientific claim with only a handful of studies behind it (particularly those with a conflict of interest in the study outcome or questions regarding the data) should always be couched in the language of “emerging evidence” rather than “conclusive findings” [3]. It is possible that some people who have stopped drinking regular milk due to digestive discomfort may be able to tolerate A2 milk, but the evidence is inconclusive. If you already drink regular milk without issues, there is no reason to switch.


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