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A1 milk and beta-casomorphin-7

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ABSTRACT

Milk is composed of water, proteins, lipids, lactose, vitamins, and minerals. More than 80% of most mammals' milk proteins are constituted by caseins. Casein is a group of proteins and they are sub-divided into αs1-, αs2-, β- and κ-casein families. Among these casein families, β-casein is the second most abundant protein. Different mutations in the cow milk β-casein gene led to 12 genetic variants and most common of these are genetic variants A1 and A2. The A1 and A2 variants differ only at amino acid position 67, which is histidine in A1 or proline in A2 milk. This difference in amino acid sequence suggests a conformational change in the secondary structure of the expressed β-casein. Milk that contains A1 β-casein and A2 β-casein are known as A1 milk and A2 milk, respectively. A1 β-casein milk releases an amino acid bioactive peptide called beta-casomorphin-7 (βCM-7) in small intestine. Beta-casomorphin-7 released from A1 β-casein is responsible for many human disorders like type-1 diabetes, autism, schizophrenia, alzheimer's disease (AD), attention deficit hyperactivity disorder (*ADHD*), multiple sclerosis (MS) and heart diseases.

Keywords: A1 Milk, A2 milk, βCM-7, Casein, Health

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Introduction

Milk is a liquid secreted from the mammary glands of female mammals following birth for the purpose of feeding the offspring. Since it is secreted with the purpose of feeding it - which after the birth is incapable of consuming any other type of food- the milk contains almost all the nutritional elements required to nourish the offspring. The milk, therefore, is a rare type of food which has the potential to contain all the nutrients necessary for life, and which the newborn offspring can consume easily (Fox et al, 2015). Although the amount of milk is very limited in humans and is secreted only in sufficient quantities for the baby, in animals it is usually secreted in higher amounts than needed by the offspring. This makes it possible to use some of the animal milk for the feeding purposes of the humankind. Furthermore, genetic studies have made certain animals capable of yielding significantly higher amounts of milk than they normally do (Patton, 2017).

While milk shows differences in composition based on the mammal species it is secreted from, its primary components are always proteins, fats, and carbohydrates. The ratio of these components changes based on the needs of the offspring of the specific mammal species it was secreted for. Milk further contains other minor components like minerals, vitamins, and enzymes. When naming it, milk is called by the name of the animal it was secreted from (Fox et al, 2017).

Milk proteins, milk fat, and lactose -which is the only carbohydrate in the milk- all hold a significant place in terms of nutrition and of the health of the consumer, and for the technological processing of the milk. Milk proteins are divided into two main parts as casein and serum proteins. A significant proportion of milk proteins are made up of caseins. Milks with casein that makes up for more than 2/3 of its total proteins are called the "casein milks", whereas milks that contain other serum proteins in similar amounts with the casein are called the "albumin milks". Milks of cows, sheep, goat, and buffalo are casein milks, while human, mare, donkey milks are albumin milks. Milks sold in markets and the majority of the dairy products that have technologically been processed are obtained from casein milks. Neither casein nor other serum proteins are of a single type of protein structure. Serum proteins are albumin, globulin, and protease-peptones. Casein, is composed of αs-casein, β-casein, κ-casein and γ-casein fractions (Mehta, 2015).

Proteins are the smallest component blocks of the body, in addition to their important roles of formation and repair of the tissues, undertaking the transportation and storage of various substances, and taking part in in the immune system functions. In the food, proteins are important elements which determine the nutritional value, textural and organoleptic properties of the food. Due to containing all the essential amino-acids, milk proteins are considered to be "total proteins" in terms of their nutritional properties (Damodaran, 1996). Proteins are also a very important source for bioactive peptides. Bioactive peptides are usually protein breakdown products with a short-chain structure containing 2 to 50 amino acids. Although they are present in many different foods like milk, eggs, beans, fish, and corn, milk proteins represent the most important source of bioactive peptides (Park and Nam, 2015). Bioactive peptides found in inactive form are created during the digestion of milk by the digestive system enzymes, during the fermentation of milk by proteolytic starter cultures, or through enzymes derived from microorganisms or plants. Milk-derived bioactive peptides have different physiological bioactivities and properties, like their antihypertension, antimicrobial, antioxidant, antithrombotic, immunomodulator, mineral binding, and opioid effects (Nongonierma and Fitz-Gerald, 2015; Mohanty et al, 2016). However, studies have shown that beta-casomorphin 7 (βCM-7), which is a bioactive peptide and forms as a result of the breakdown of beta-casein, has multiple negative effects on health.

β-Casomorphin7 (βCM-7)

Approximately 82% of cow's milk proteins consist of caseins and 30-35% of the casein consists of β-caseins. So far, 12 β-casein genetic variant has been identified. These are the A1, A2, A3, B, C, D, E, F, H1, H2, I and G variants. Amongst these, A1, A2, A3, and C are commonly encountered in dairy cattle, and the most common are A1 and A2 variants. Whether a cow will have A1 and/or A2 variant is determined by a couple of genes located in the 6th chromosome. This particular gene has 2 alleles, known as A1 and A2 β-casein alleles. Research has shown that each cow carries two copies of β-casein genes. It is possible for a cow to have any one of the A1A1 (homozygote), A2A2 (homozygote), or A1A2 (heterozygote) alleles. In case the animal has the A1A2 alleles, none of these alleles are dominant over the other, and the milk contains equal amounts of A1 and A2 β-casein. For this reason, a cow with A1A2 produces equal amounts of A1 and A2 β-casein, while a cow with A2A2 genes produces the A2 β-casein alone and a cow with A1A1 genes produce only the A1 β-casein (Priyadarshini et al, 2018). Whether the casein is A1 or A2 is based on a small variation in the amino-acid sequence. The 67th amino acid is histidine in A1 β-casein, whereas it is proline in A2 β-casein. The peptide bond between the 66th and 67th amino acids in A1 β-casein, which are isoleucine and histidine, can be broken down by elastase, whereas the same bond for the A2 β-casein (between the isoleucine and proline in this case) can't be broken down by elastase (Figure 1). Such a breaking down of this bond for the A1

β-casein results in the formation of β-casomorphin 7 (βCM-7), whereas it results in the formation of β-casomorphin 9 (βCM-9) for the A2 β-casein. Considering this, milks that contain the A1 variant are called A1 milks, and the ones with A2 variant are called the A2 milks (Nguyen et al, 2015).

A1 milk, and therefore the βCM-7, has been reported to affect countless opioid receptors in nervous, endocrine, and immune systems potentially, and to represent a risk factor for a variety of important diseases and disorders like cardiovascular diseases, type-1 diabetes, autism, and schizophrenia. A2 milk, on the other hand, represents no such risks (McLachlan, 2001; Kaminski et al, 2007; Chia et al., 2017; Banerjee, 2018). There is a predominant A1 β-casein variant (A1/β-casein = 0.46-0.71) in the milk of red-white (Red Danish, Holstein-Friesian, Ayrshire) and black-and-white cattle breeds found in northern Europe (Buchberger, 1995). As a result of artificial insemination practices between 1970 and 1980 using the American Holstein bulls, the level of the A1 variant in the milk of northern European cattle has declined. The A1 variant level in the central and southern European milks is low as well. This is due to the fact that breeds like the Guernsey which has a low level of A1 variant (A1/β-casein ratio mostly <0.25) and Jersey Simmental Swiss Brown which almost doesn't have any A1 variant (A1/β-casein = 0.01) are the predominant races in the region (Zikakis et al, 1974). The results of the studies performed to this date have shown that milks from Northern European cattle breeds such as the Friesian, Ayrshire, British Shorthorn and Holstein breeds contain A1 β-casein, while milk from Channel Island cattle, Guernsey, Jersey, southern France domestic cattle, Charolais - Limousin, African original Zebu cattle, and Gir, Tharparkar, Rathi, Red Sindhi, Sahiwal, Kankrej and Hariana dairy cattle of India contain A2 β-casein. Milks from African and Asian pure indigenous breeds contain only A2 β-casein and is called the "safe milk" (Laugesen and Elliott, 2003; Boro et al, 2016).

βCM-7 is a biomodulator substance, and is considered as an exorphine. βCM-7 is a μ-Opioid receptor antagonist that is capable of entering the blood circulation through the digestive system. Its amount in the circulation increases with the amount of the consumed milk. Human milk especially colostrum contains this exorphine as well, but its activity in human milk is lower compared to the cow milks. Also human milk β-casein structure is the closer to cow A2 milk β-casein structure than cow A1 milk β-casein (Jarmołowska et al, 2007; Ul Haq et al, 2014a; Chia et al, 2017; Priyadarshini et al, 2018) (Figure 2). βCM-7 has also been detected in the blood of pregnant and breastfeeding women, but it hasn't been determined in males and in non-pregnant women. Accordingly, βCM-7 has also been emphasized to potentially have a physiological significance for pregnancy and for birth (Jaiswal et al, 2014). The 4th and 5th position amino acids of βCM-7 of cow and human milk are different from each other. Due to this structural difference, the opioid effects of βCM-7 originating from these two sources is also different. βCM-7 of cow origin has 10 times more opioid effect compared to its human-origin counterpart.

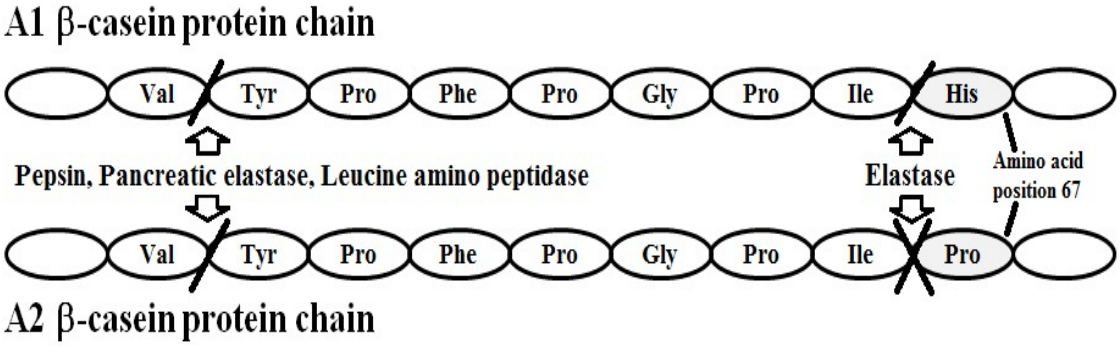

Figure 1. Difference between conformational change of A1 β-casein and A2 β-casein

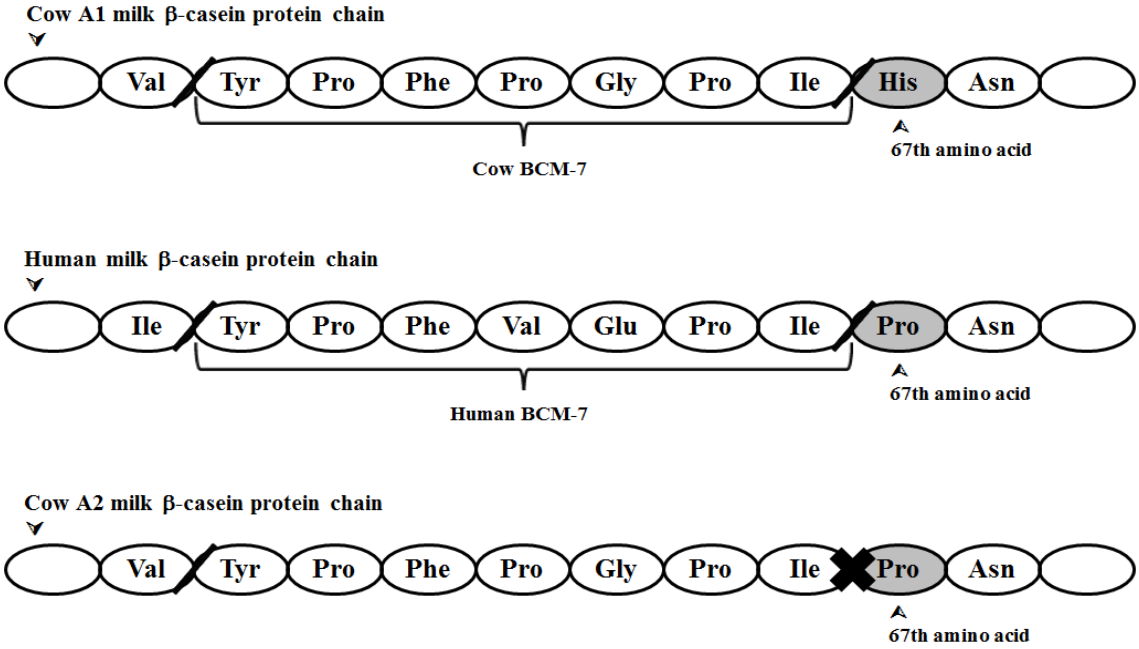

Figure 2. Structure of cow milk A1 and A2 β-casein, human milk β-casein, cow βCM-7 and human βCM-7

Studies have shown that cow βCM-7 may play a neuro-chemical role in establishing the mother-cub connection, and in learning and environmental adaptation processes of calves and young cattle. Accordingly, βCM-7 doesn't represent a health risk for the individuals that consume the milk from their own species -on the contrary, it has certain positive roles to play-whereas the βCM-7 originating from A1 dairy milk, in particular, can cause a series of health problems for humans (Jaiswal et al, 2014). βCM-7 was determined in whole pasteurized milk, ultra-heat treated milk, bottle-sterilized milk, fermented milks like yoghurt and probiotic fermented milk (Ul Haq et al, 2015). But it is reported that fermentation and storage reduced βCM-5 and βCM-7 concentration in yoghurt (Nguyen et al, 2014; Nguyen, 2015).

βCM-7 and Health

Since they contain all of the essential amino acids in their structure, milk proteins are very important in terms of nutrition for people of all ages. The human being, who starts life mostly by consuming breast milk, also begins to consume milk from various animals and dairy products obtained from these in his childhood and adulthood periods. Consumption of milk proteins was shown to reduce the risk of various diseases like diabetes, muscle atrophy, atherosclerosis, high blood pressure, cardiac diseases, and osteoporosis, and to further have anticarcinogenic, hypocholesterolemic, and ACE (angiotensin-converting enzyme) inhibitory effects through bioactive peptides within the milk (Davoodi et al, 2016; Mohanty et al, 2016; Banerjee, 2018; Lorenzo et al, 2018). That being said, one particular bioactive peptide called βCM-7, which is one of the hydrolysis products of casein, can act as a risk factor for various diseases and disorders like type-1 diabetes, ischemic heart disease, autism, schizophrenia, and sudden infant death syndrome (Kadam et al, 2017; Banerjee, 2018).

Type-1 Diabetes

There are claims that there is a positive correlation between the consumption of A1 milk and the incidence of Type-1 diabetes. The relationship between Type-1 diabetes and A1 milk consumption is explained by several theories. According to the first of such theories, βCM-7 suppresses the immune system of the individual and increases the vitality of enteroviruses, endogenous retroviruses and/or pathogens bacteria such as Mycobacterium avium which then damage the pancreatic β-cells. Increased vitality of these types of pathogens is related to the triggering of symptoms akin to type-1 diabetes in the individual (Kaminski et al, 2007; Parashar and Saini, 2015; Chia et al, 2018). Another theory holds that certain peptides that are hydrolysates of β-casein mimic the structure of GLUT-2 protein, which is normally tasked with the transportation of the glucose. Sensing these

peptides as antigens, the T-cells activate the beta cells to produce antibodies against them. In the end, these antibodies destroy not only the β-casein variants but also the beta cells that produce the insulin, resulting in type-1 diabetes (Parashar and Saini, 2015).

Epidemiologic studies related to the subject have shown strong positive correlations between type-1 diabetes and A1 milk consumption. In a 15-year study that covered 19 countries -including Finland, Austria, Iceland, Denmark, and France- a very strong correlation between the proteins originating from A1 milk (with the exclusion of cheese) and occurrence of type-1 diabetes was revealed ($r=0.92$, $p<0.00001$). The same study reports that A1 milk consumption for the periods of 0-4, 5-9, and 10-14 years and incidence rate of type-1 diabetes as $r = 0.80$, 0.81, and 0.81 respectively, which are quite similar to each other. The study has reported the correlation between the type-1 diabetes occurrence and consumption of A2, B, and C variants of casein was insignificant. It was also revealed by that particular study that occurrence of type-1 diabetes was higher in countries like Finland and Sweden where A1 consumption per individual was high, whereas it was lower in countries like Japan and Venezuela where A1 β-casein consumption was low (Laugesen and Elliott, 2003).

The results obtained from animal experiments also show meaningful relationships between A1 β-casein consumption and type-1 diabetes incidence. In a study performed on non-obese diabetic mice, none of the mice fed with A2 β-casein diet developed auto-immune diabetes, whereas 47% of the mice fed with A1 β-casein diet developed auto-immune diabetes (Elliott et al, 1997). Another study has shown that 50% of the rats fed with a standard laboratory feed developed auto-immune diabetes, while this ratio dropped to 15% in rats which were fed with a semi-synthetic diet. Introducing milk to the semi-synthetic diet, however, has increased the ratio back to 52% (Elliott and Martin, 1984).

Knip et al. (2014) reported that being fed with mixed diet proteins increases the type-1 diabetes risk for children that are genetically predisposed. Beta-cell autoimmunity emerges in the early periods of life, and the diets consumed in these periods may alter the type-1 diabetes risk. Case assessment studies on the subject have shown that the consumption of cow milk from early periods of childhood is a risk factor for type-1 diabetes (Gimeno and de Souza, 1997; Virtanen et al, 2014). For infants, consumption of cow milk before 2 months of age has been reported to be a more influential environmental factor for type-1 diabetes, compared to milk consumption after 4 months (Knip et al, 2010a). A study performed in Finland has shown, based on the data obtained from a total of 690 children with type-1 diabetes and less than 15 years of age, that consumption of cow milk proteins before 2 months of age increased type-1 diabetes risk by twofold (Virtanen et al, 1993). Some researchers report that cow milk proteins increase the type-1 diabetes risk in genetically predisposed children (Knip et al, 2010b; Chia et al, 2017) while others have reported that cow milk is a risk factor for type-1 diabetes regardless of genetical predisposition (Lamb et al, 2015).

Ischemic Heart Disease

Epidemiological studies have shown that consumption of β-casein A1 has a strong relationship with ischemic heart disease. Despite consuming animal milk in great amounts, Masai communities of East Africa and Samburu communities of Northern Kenya have little to no incidence of heart disease. This is because these communities obtain their milk from the zebu cattle, which carry the A2 alleles (McLachlan, 2001). In a study performed covering a total of 17 countries, the amount of A1 milk consumption in 1980 and the incidences of death caused by cardiovascular diseases in the years of 1985 and 1990 was found to have a strong correlation. In another study performed regarding the cow milk and ischemic heart diseases, the correlation between the A1 β-casein amount consumed per individual and the ischemic heart disease was found as $r=0.76$, which is considerably high. The study took into account diseases that have occurred 5 years after the consumption (Laugesen and Elliott, 2003). Similarly, was reported a strong correlation ($r=0.86$) between the consumption of A1 β-casein of milk protein origin (excluding the cheeses) and deaths due to ischemic heart diseases [WHO MONICA (monitoring trends and determinants in cardiovascular disease) project] (McLachlan, 2001).

An animal experiment has seen rabbits fed with diets containing different ratios of casein variants (A1 and A2), serum proteins, and cholesterol. Comparison of diets that didn't include cholesterol in their diets has revealed that diets that contained β-casein A1 have resulted in higher serum cholesterol, LDL, HDL, and triglyceride levels, compared to the diets that contained β-casein A2 and serum proteins (Tailford et al, 2003). It has also been reported that total casein consumption (which includes all the sub-fractions of casein obtained from milk through acid precipitation) has promoted the development of atherosclerosis in rabbits, monkeys, and mice (Anthony et al, 1998; Ni et al. 1998) and that β-casein A1 was atherogenic (Tailford et al, 2003).

Tyrosyl is a protein oxidation product and was determined to be present in the atherosclerotic lesions, and βCM-7 is a potential source for the tyrosyl radical (Zeng et al, 2018). Furthermore, βCM-7 has physiological effects over the oxidation

of LDL, and peroxidation of LDL lipid components. These oxidation products, in turn, may cause the development of various heart diseases (Kamiñski et al, 2007).

Autism and Schizophrenia

Autism is a lifelong neurodevelopmental disorder that often affects social, cognitive, and creative abilities (Shattock and Whiteley, 2002; Crane et al, 2016). Even though certain genes that can cause autism have been identified, it is claimed that genetic and environmental factors both can play a role in the development of the disorder (Shattock and Whiteley, 2002; Abrahams and Geschwind, 2008; Sokolov et al, 2014). The fact that certain milk protein-derived peptides have an opioid effect has led to the development of the hypothesis that a relationship between autism and extreme opioids may exist. According to this hypothesis, genetic disposition and/or environmental stress at early ages can cause changes in the intestinal functions, an increase in the permeability of the intestinal mucosa, and cause a reduction in proteolytic activity. Together with these factors, reduced peptidase activity and increase in blood-brain barrier may result in accumulation of opioid peptides (like casomorphin) in the brain and in the blood, which in turn may result in hyperpeptidemia. In the end, the chronically increasing exorphine levels in the brain may affect the opioid and neurotransmitter systems, causing the emergence of disorders like autism (Shattock and Whiteley, 2002; Sokolov et al, 2014).

According to the data obtained from infants, fed breast milk and baby food containing cow's milk, the highest human βCM-7 immune-reactive substance concentration was found in the infants fed breast milk, whereas the highest concentration of cow βCM-7 immune-reactive substances were found in infants fed with baby food containing cow's milk. The babies fed breast milk displayed normal motor and muscular development, whereas the babies fed with baby food containing cow's milk had delayed motor and muscular development (Kost et al, 2009).

Autistic children and their mothers were found to have high amounts of endogenous opioid peptides in their serum, blood cells, and cerebrospinal fluids (Brambilla et al, 1997; Nagamitsu et al, 1997; Leboyer et al, 1999; Tordjman et al, 2009). In a study, the urine of autistic children was found to contain higher βCM-7, compared to the urine of healthy children in the control group. The study has reported that the βCM-7 presence and the autistic symptoms were correlated, and continuous βCM-7 consumption in early ages provided a step for autistic disorders, damaging the early development period of the child (Sokolov et al, 2014). Animal experiments have shown that βCM-7 may play part in developing behavioral disorders similar to those seen in autism and schizophrenia (Sun and Cade, 1999; Sun et al, 1999).

Schizophrenia and autism are related to hyperpeptemia and hyperpeptidurea, and it has been reported that elimination of the low molecular weight peptides from the blood through hemodialysis, or feeding with diets that don't have milk or gluten, could result in remission of schizophrenic symptoms. Also reports that 90% of the schizophrenic patients and 86% of the autistic patients had high βCM-7 IgG antibodies (Cade et al, 1990).

Sudden Infant Deaths

Sudden Infant Death Syndrome refers to the death of the infants of 12 months of age and lower, usually in their sleep. Brain anomalies, lower birth weight, respiratory infections, and environmental factors that prevented the baby from breathing can be cited amongst the potential causes of death. It has been claimed that βCM-7 is also amongst the causes of death (Mallepalli et al, 2017; Sun et al, 2003). βCM-7 is quite stable against enzymatic breakdown. Yet, it is the substrate of Dipeptidyl-peptidase IV (DPPIV). After an apnoea event, infants with apnoea were found to have higher levels of βCM-7 and lower levels of DPP4 in their serum compared to those who were healthy. Accordingly, it has been reported that the high concentration of βCM-7 due to increased DPPIV levels could be responsible for the depression of respiration (Wasilewska et al, 2011). βCM-7 taken in by the diet can be absorbed through the digestive tract and can pass the blood-brain barrier due to the central nervous system not being fully developed yet. In babies with abnormal breath control or with vagal nerve development disorders, βCM-7 causes depression in the respiration center in the brain, causing death (Sun et al, 2003).

Other Claims Regarding A1 Milk and βCM-7

A subject being discussed is the calcium/magnesium ratio differences between A1 and A2 milks. A1 milks have calcium to magnesium ratio of 10:1, whereas A2 milks have a ratio of 2:1. Extended periods of A1 milk consumption may lead to magnesium deficiency and the problems associated with it (Boro et al, 2016).

In some studies, results were obtained related with both inflammatory and immune responses to casomorphins within the gastrointestinal system. In a study where mice were fed orally with βCM-7 or βCM-5, it is reported that both peptides increased expression of inflammatory markers. The authors reported that βCM-7 and βCM-5 stimulate inflammatory responses through the T2 pathway (Ul Haq et al, 2014a). Ul Haq

et al. (2014b), reported similar gastrointestinal immune effects in mice fed a milk-free basal diet supplemented with A1 compared to mice fed a diet supplemented with A2 β-casein.

Another subject being discussed is the βCM-7 and lactose interactions. There are tree mechanisms that affect lactose malabsorption. The first mechanism is that the βCM-7 affects the activity and production of lactase with its inflammatory characteristics. The second mechanism is that changes in the gut microbiota caused by gut inflammation affects the processing of malabsorbed lactose. The third mechanism is that delayed gastrointestinal transit caused by βCM-7 leads to increased lactose fermentation (Pal et al, 2015). In their study using rats, Barnett et al. (2014), have shown that consumption of A1 β-casein has direct effects on gastrointestinal function and A1 β-casein diets delay gastrointestinal transit time compared to A2 β-casein diets.

Conclusions

A1 and A2 milks that contain the A1 and A2 variants of β-casein are still discussion topics in terms of public health. Based mostly on epidemiologic and animal-based experiments, A1 milk and the βCM-7 are claimed to cause various diseases and disorders like type-1 diabetes, ischemic heart disease, autism, schizophrenia, magnesium deficiency, and sudden infant death syndrome. Data from human clinical trials to back these claims up are limited. That being said, the claims are serious enough, considering the diseases and disorders attributed to A1 milk and βCM-7 as potential causes. For this reason, the subject has to be researched with additional expemental studies.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Ethics committee approval: The authors declare that this study does not require ethical permission.

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