Milk is a basic food for infants and children and a common food for adult humans all over the world. It has been regarded as nature's perfect food, providing an important source of nutrients including high quality proteins, carbohydrates and micronutrients. Cow milk contains about 87 percent water and 13 percent solids comprised of proteins, fats, lactose and minerals. More than 95 per cent of the cow milk protein contains caseins and whey proteins. Among the caseins, beta-casein is the second most abundant protein and has excellent nutritional balance of amino acids. Different mutations in bovine beta-casein gene have led to 12 genetic variants—A1, A2, A3, B, C, D, E, F, H1, H2, I and G out of which A1 and A2 are the most common. Milk containing A1 beta-casein is generally called as A1 milk while that containing A2 beta-casein is called as A2 milk. A1 and A2 beta-caseins are made up of a chain of 209 amino acids and the sole difference between them occurs at 67\textsuperscript{th} position in the chain where histidine occurs in A1 protein while proline in A2. Enzymatic hydrolysis of peptide bond between positions 66 and 67 releases beta-casomorphin-7 (BCM-7) from A1-beta-casein, but hydrolysis is prevented in A2 beta-casein at that location, so BCM-7 is not released from A2 proteins. It is claimed that in adult humans BCM7 is responsible for very high incidence of Heart related diseases, Diabetes, Ulcerative colitis, Multiple sclerosis, Parkinson, Schizophrenia etc. and in infants it is associated with Autism, Diabetes type 1 and Sudden death Syndrome. However, more data on animal as well as human trials is required to support the A1/A2 hypothesis. This paper briefly reviews various findings related to A1 and A2 types of milk.

EFFECTS ON HUMAN HEALTH

The research was started in the 1980s, to explore whether some peptides including peptides from casein that are created...
during digestion have negative or positive health effects. Epidemiological research and animal studies conducted in the early 1990s by scientists in New Zealand found correlations between the consumption of milk with A1 beta-casein proteins in some countries and the prevalence of various chronic diseases in those countries. A company, A2 Corporation, was founded in New Zealand in 2000 to commercialize a genetic test to determine whether a cow will produce milk without the A1 protein, and to market "A2 Milk" as a premium milk that is less risky due to the lack of peptides from A13. The review report published by European Food Safety Authority (EFSA) in 2009 concludes that experiments in cells and animals have shown that BCM-7 can act as a weak opioid receptor agonist, but that in most of the animal studies, BCM-7 was not administered orally, as humans would be exposed to it, but rather was given to animals by injection into the peritoneal cavity or even directly into the spinal cord or brain, which makes these studies not useful for understanding how BCM-7 might affect humans. The EFSA also found no relationship between chronic diseases and drinking milk with the A1 protein. The EFSA study emphasized the dangers of drawing conclusions from correlations identified in epidemiological studies and the dangers of not reviewing all the evidence at hand. No demonstration was found showing evidence of diabetes caused by consuming milk with A1 casein. A 2014 review of research pertaining to the relationship between consumption of dairy products (including A1 and A2 proteins) and the incidence of diabetes revealed a positive correlation between consumption of dairy products by babies and the incidence of type 1 diabetes in some people, whereas an inverse relationship between the consumption of dairy products and the development of type 2 diabetes in some people, these correlations were tentative. It was impossible to determine what component or components of milk might be responsible for these effects, and it is unlikely that the expensive and complex research to determine the components of dairy products by babies and the incidence of type 1 diabetes in some people, whereas an inverse relationship between the consumption of dairy products and the development of type 2 diabetes in some people, these correlations were tentative. It was impossible to determine what component or components of milk might be responsible for these effects, and it is unlikely that the expensive and complex research to determine the answers to these questions will ever be conducted. 

**Type I diabetes mellitus (DM-I):** Positive associations between DM-1 incidence and milk consumption and negative associations with breastfeeding have been shown in epidemiological studies, but not all studies have found these relationships. Evidence that milk consumption is related to DM-1 has been summarised by Schrezenmeir and Jagla and Pozzilli. Elliott noticed the fact that there was very low incidence of DM-1 among children in Polynesian islands compared with Polynesian children in Auckland. Prolonged breast feeding appears to be protective against DM-I. Cow's milk antibodies were found at higher levels in diabetic children. DM-I rates between countries correspond fairly well with cow's milk intake. DM-I is rare in children of the Masai in E. Africa, a tribe with high consumption of milk from Zebu cows (Bos indicus). Elliott et al., collected published data for diabetes incidence in children (0-14 y) in 10 developed countries and calculated the consumption of the total milk protein and of A1 and B beta casein. They used FAO data for national milk protein consumption, information and breed composition of the cows and their milk protein polymorphism. They found that total milk protein did not correlate significantly with DM-I but A1 beta casein did and correlation was even stronger with A1 and B beta casein.

BCM-7 has opioid and cytomodulatory properties. Synthetic BCM-7 can inhibit responses of lymphocytes to stimulants in vitro. Elliott et al., reported that non-obese diabetic (NOD) mice fed A1 beta casein did not develop diabetes if they were also given naloxone (the morphine antagonist). The antibody response to ovalbumin was prevented in NOD mice if they were also given injections of (synthetic) BCM-7; this prevention did not happen in Swiss mice. They suggested that appearance of diabetes in genetically susceptible NOD mice fed A1 beta casein and not in those fed A2 beta casein might be due to release of the bioactive peptide, BCM-7 from A1 beta casein, which had a strong inhibitory effect on immune function.

The study by Padberg et al., showed that antibodies against A1 beta-casein are increased in patients with DM-1. Immune responses to a variety of other antigens (e.g., GAD, BSA, beta lactoglobulin, α-casein) were also tested. In general, patients with DM-1 had higher levels of immune response to beta-casein, but the overlap with controls was substantial and responses to other antigens were also often increased. There was a considerable debate about whether this evidence of immune activity was linked to the etiology of DM-1 or was a consequence of the disease process.

Several feeding studies have been undertaken with a variety of animal models of DM-1. Often a pre-hydrolyzed casein-based infant formula is used as a diabetes protective diet. A cereal-based diet (mainly wheat, corn and soybean) called NIH-07 is
often used as a standard diabetes-promoting diet. Two animal studies specifically tested the A1 / A2 fractions. Studies reported by Beales et al. used 2 animal models and 9 different diets in 3 centers internationally. The most diabetogenic diet was a base diet with milk protein free composition. This caused diabetes in 71 per cent of NOD mice and 73 per cent of BB rats. The base diet was either Prosobee (soy isolate) or Pregestamil (hydrolyzed casein) and this resulted in diabetes rates between 17 per cent and 39 per cent in both animal models. Overall, the addition of 10 per cent of either A1 beta-casein or A2 beta-casein made no difference to the diabetes rates except in the BB rat where Prosobee+A1 caused more diabetes than Prosobee+A2 (46 per cent versus 19 per cent, p<0.05). These findings are in contrast to the earlier study reported by Elliott et al.. Their findings were much more clear-cut showing a marked increase in diabetes incidence with the A1 compared to the A2 supplemented diet. The negative results of the multi-centre study appear to be scientifically more robust than the earlier positive study by Elliott et al., .

Further studies will be needed to resolve the susceptibility of animal models for DM-1 to diets with variations in A1 / A2 beta-casein content.

No longitudinal studies or feeding trials in humans have been reported on the roles of A1 and A2 beta-casein in the etiology of DM-1. Such studies are possible (especially within genetically at-risk infants) and would contribute substantially to the evidence base.

**Cardiovascular Diseases:** The contention of the A1/A2 hypothesis is that a high intake of A1 beta-casein is a risk factor for IHD. There is some evidence that animal proteins are more cholesterolemic and atherogenic than plant proteins. Several animal models (rabbits, monkeys, mice) have shown that high casein consumption promotes atherosclerosis. CNS McLachlan, produced evidence for a correlation of mortality from CHD in 16 countries with national A1 beta-casein consumption (g/day) and applied for patents in New Zealand and with the WTO. Contending that consumption of milk protein beta casein A1 promotes the development of heart disease in humans. A rabbit experiment was reported in which rabbits killed after feeding with 10 per cent A1 beta casein for 6 weeks showed larger areas of aortic fatty streaks than animals that received A2 beta casein. Serum cholesterol was higher in the A1 than the A2 beta casein group of rabbits. More extensive correlation calculations of A1 beta casein and other dietary variables against DM-I and CHD were published by Laugesen & Elliott. They concluded: Cow A1 beta casein per capita supply in milk and cream (A1/capita) was significantly and positively correlated with ischemic heart disease (IHD) in 20 affluent countries five years later over a 20 year period, providing an alternative hypothesis to explain the high IHD mortality rates in northern Europe compared to southern Europe. Surveys of A1 beta casein consumption in two-year-old Nordic children, and some casein animal feeding experiments, confirm the A1/capita and milk protein/capita correlations. They raised the possibility that intensive dairy cattle breeding might have emphasized a genetic variant in milk with adverse effects in humans. Further animal research and clinical trials would be needed to compare disease risks of A1-free versus ‘ordinary’ milk.

**Schizophrenia and Autism:** The hypothesis that linked neurological disorders such as schizophrenia and autism to A1 beta-casein was that, in genetically susceptible individuals, casein was cleaved in the gut to produce casomorphins, a peptides with opioid characteristics. These compounds enter the circulation, cross the blood-brain barrier and influence neurological functioning. BCM-7 has long been considered a risk factor for autism but the hypothesis remains controversial. Trials with animals showed that BCM-7 crossed the blood-brain barrier and led to autistic type behaviour. Milk elimination trials in humans have produced positive results but were often criticized for lack of double blind protocols.

Bovine BCM-7 has been detected in the blood of human infants by two different research groups using immunoassay methods, and bovine BCM-7 has also been detected in the urine of children with autism spectrum disorders using mass spectrometry analysis. A broad range of studies from American and European investigations has shown reduction in autistic and schizophrenic symptoms with decrease in A1 milk intake. But it needs to be established that whether opioids from beta-casein cause the syndromes of autism and schizophrenia or whether they are causing or exacerbating the symptoms.

Eight trials of casein-free diets in people with autism have been published which have generally been of poor scientific design with no control group or blinding of measurements.
Seven of the trials were uncritically reviewed by Knivsberg et al., (2001) although taking the study biases into account; they were suggestive of an improvement in several autistic behaviours and functioning with casein-free, gluten-free diets. The best study was the most recent one (Knivsberg et al.,) which had a better design with blinded assessments where possible and a random allocation to diet or no diet. The majority of the measurements showed significant improvements on the diet (casein-free, gluten-free).

**Effects on Digestive system:** Bovine BCM-7 when released in the gut can affect the digestive system and like other opioids can reduce the rate of passage through the gut, causing constipation. BCMs are mu-type opioid agonists and mu-receptor activation is known to produce constipation. Zogbhi et al., examined the in vitro effects of BCM-7 on human colon goblet-like cells (HT29-MTX cells) and found that BCM-7 increased mRNA levels of the mucin MUC5AC (219 per cent after 24 hours of stimulation) and the secretion of this mucin (169 per cent of controls), dependent on mu-opioid receptor activation. BCM-7 may also increase problems of lactose intolerance as transit is slow with more fermentation occurring. BCM-7 has been shown to increase inflammatory activity of colonic immune cells and mucous production and thickening in the rat small intestine. Researchers are looking at the potential role BCM-7 may have in Ulcerative Colitis, Crohn’s disease and other autoimmune conditions such as Multiple Sclerosis and Parkinson’s Disease.

**Child development:** Russian scientists have shown that BCM-7 enters the blood of babies fed milk formula diets. Whereas, some babies can quickly metabolize the BCM-7, others are slow metabolizers. In babies whose BCM-7 levels in the blood stay high between feeds, there is a high risk of delayed psychomotor development.

**Sudden Infant Death Syndrome (SIDS):** For more than 20 years BCM-7 has been suspected as a risk factor for SIDS. Casomorphins have been found in the brainstems of children who have died from SIDS but comparisons with normal children were obviously not possible. Until recently, direct evidence of apnoea-inducing effects was only available from animals. However, specific evidence of BCM-7 causing respiratory depression in humans has shown that babies who suffer acute life threatening events (ALTE) through apnoea were characterised by circulating levels of BCM-7 that were three times higher than in normal children. These children had DPP4 levels (the enzyme that degrades BCM-7) 58±3 per cent of those in normal children. The evidence was that even if the babies were breast-fed, bovine BCM-7 was still found in the blood of the infants. This suggests that bovine BCM-7 was transferring from mother’s stomach to her infant via milk. Other work by this group has found bovine BCM5 (a stronger opioid which is derived from BCM-7) in the blood of breast-fed children.

**Intolerances:** Many people who drink A2 milk do so because they find it is easier to digest. However, A2 milk does contain lactose, which is often stated as the most important milk intolerance issue. The likely explanation for this apparent contradiction is twofold. First, the BCM-7 that is released from A1 beta-casein slows down the passage of food through the digestive system providing longer time for lactose fermentation. Second, many people are intolerant specifically to the BCM-7. A simple test to investigate whether someone who is intolerant to ‘ordinary’ cows’ milk will be able to drink ‘A2 milk’, is to ask them whether they can tolerate goats’ milk. If the answer is ‘yes’, then they can also tolerate A2 milk.

**Mild allergies:** An allergy is an immune-based condition defined by The National Institute for Allergy and Infectious Diseases (NIAID) as “an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food”. In contrast, intolerance does not involve an immune response. In practice, they often run together, with the intolerance apparently being a consequential response. Mild allergies associated with BCM-7 can include eczema and asthma, with much of the evidence being case-related. BCM-7 is also known to induce production of mucins and this provides a logical explanation for why many people associate milk with mucus production. Of course both A1 and A2 milk contain a range of proteins unrelated to BCM-7 which can cause severe reactions including anaphylactic shock in susceptible people.

**CONCLUSION**

There is huge controversy among the scientific community over the hypothesis that a high intake of milk containing A1 beta-casein promotes conditions like DM-1, IHD, schizophrenia.
and autism. But the hypothesis is potentially important and cannot be ignored as evidence from ecological studies for DM-1 and IHD shows that there is certainly a possibility that the A1/A2 composition of milk may be a factor in the etiology of these conditions which needs to be supported by the scientific human trials. The evidence in relation to autism came mainly from poorly controlled clinical trials of gluten-free, casein-free diets where some improvement was noted in the autism characteristics and behaviours. The evidence in relation to schizophrenia and other disease conditions is very minimal.

The A1/A2 hypothesis is interesting and potentially very important for public health if it is proved correct. It should be taken seriously and deeper research is needed to verify the range and nature of BCM-7 interactions with the human gastrointestinal tract as well as all other body systems. This requires more of animal trials and generation of data on human subjects having the problems related to A1/A2 beta-casein milk consumption.

REFERENCES


