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A1 and A2 beta casein: Twin faces of milk

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Abstract
Proteins in bovine milk are common source of bioactive peptides. Components in milk show various constructive actions but some of the studies found that metabolites of it show severe threat to human health. The BCM-7 peptide which is released by gastrointestinal proteolytic digestion of bovine beta-casein variants A1 and B but not from A2 posses a deleterious effect on health. Epidemiological evidences proposes that consumption of beta-casein A1 is associated with higher mortality rates from Ischemic heart disease, cardiovascular diseases and type 1 diabetes and even neurological disorders, such as autism and schizophrenia. Therefore this review aims at enlighting the differences between the consumption of milk containing A1 and A2 type beta casein and its effects on human health.

Keywords: beta-casein, beta-casomorphin-7, CVD, DM-1, autism, A1, A2

Introduction
Milk is reflected as one of the crucial foods all over the world, providing an imperative source of nutrients including high quality protein, carbohydrates and particular micronutrients. Being rich in these constituents, milk has been gazed as nature's flawless food. Milk is an emulsion of oil in water, comprising of 87.7% water, 3.3% protein, 4.9% lactose, 3.4% fat, 0.70% minerals and 3.36% minor components. Milk contains various types of protein, out of which predominantly two are considered imperative, Whey protein (14%) (Roginski. 2003) [23] and Casein (80%) (Niki *et al*., 1994; Martien *et al*., 1994) [22, 18]. Casein is a composite of several components and is the predominant class of proteins in milk. There are four chief subgroup of Casein, αS1 Casein (CSN1S1, 39–46% of total caseins), αS2 Casein (CSN1S2, 8–11%), β Casein (CSN2, 25–35%), κ Casein (CSN3, 8–15%) (Roginski. 2003) [23], which are all heterogeneous and consists of several genetic variants. Among Caseins, β Casein is the second copious protein fraction in cow milk. In the recent past, there is a budding public health concern, particularly regarding milk. Milk despite being perfect food, some of elements in cow's milk is triggering problem to human beings. Due to mutations in β Casein gene during the course of evolution have led to 13 known variants out of which two major variants i.e., A1 and A2 are usually found in milk out of which neither A1 nor A2 traits seems to be dominant (co-dominant). When assessment is made between these two types of variants, chief difference is the positioning of amino acids in their chain. An inventive proposition was developed during 1990's by few researchers, that a protein in the milk of some cows, not others is an risk factor, which on consuming leads to type 1 diabetes (DM-1), coronary heart disease (CHD), gastrointestinal discomforts, neurological disorders, sudden infant death syndrome (SIDS), autism etc. (Laugesen and Elliott, 2003) [17]. Now the query facing us is to choose whether to guzzle milk? If yes, then, milk containing A1 variant or A2 variant!

History of A1 and A2 milk
The beginning for the quest to quench the mystery about A1 and A2 started during 1993 in New Zealand by Professor Bob Elliott from Auckland University during his epidemiological survey regarding incidence of Type 1 diabetes among Samoan children. He was aware of the fact that Samoan children in New Zealand were susceptible to Type 1 diabetes, but incidence of the same in Samoan children residing in Samoa was extremely low. So Prof. Elliot suspected that, answer might be related to or around consumption of milk, which was much lower in Samoa. Along with Dr. Jeremy Hill from New Zealand Dairy Research Institute he started working on mice that had been specially bred for susceptibility to diabetes. Where initial results revealed difference in the diabetes incidence between those fed A1 β Casein and those fed with A2 β Casein. None of the mice fed with A2 β Casein were found diabetic but on the other hand 47% of mice fed with A1 β Casein were diabetic after 250 days.

A2 β Casein was primarily found in cows even since before humans started domesticating those. But a mutation might have occurred about few thousand years ago, which gave rise to a fraction of cows of European breeds producing a casein variant called A1 beta-casein. This mutation has successively been blowout widely throughout herds in the western world. Nevertheless, there is substantial metamorphosis in the predominance of the A1 gene among breeds, countries, and in some cases, provinces.

β- Casein

A1, A2, A3, B, C, D, E, F, H1, H2, I, G are the genetic variations of beta-casein (Kaminski *et al*., 2007) [6]. A1 and A2 are the most collective forms of beta casein in cattle breeds, while B is fewer amount, and A3 and C are very rare (Farrell *et al*., 2004) [8]. In 67th position of the beta-casein chain, Histidine in A1 variant is switched by proline in A2 variant (Roginski. 2003) [23]. Recent awareness on milk containing A1 beta-casein is that histidine at the 67th amino acid position permits digestive enzymes (pepsin, pancreatic elastase, leucine aminopeptidases) (Elliott *et al*., 1999) [7] to chop out a 7 amino acid segment of that protein just adjacent to the histidine, on the other hand, proline at the same location in A2 beta-casein, enzymatic hydrolysis of the Ile^{66} –Pro^{67} occurs at very low rate or not at all. The natural mutation that gave rise to this difference is a result of single nucleotide polymorphism at codon 67 of the beta-casein gene: CCT (A2 proline) to CAT (A1, histidine) (Kaminski *et al*., 2007) [6]. Elastase seperates the peptide bond between Ile and His, releasing the carboxyl terminus of this peptide (Jinsmaa and Yoshikawa. 1999) [10]. The 7 amino acid segment that is unglued from A1 β casein is known as Beta casomorphin-7, frequently abbreviated as BCM-7 (Kostyra *et al*., 2004) [13]. Both variants A1 and A2 are the most common in the popular dairy cattle worldwide, i.e. Holstein-Friesian.

Beta-casomorphins

Beta- Casomorphins (BCMs), the peptides originating from beta-casein, are a group with a chain length of 4–11 amino acids (aa), all starting with tyrosine residue in position 60 (Kostyra *et al*., 2004) [13]. Meisel and Fitzgerald (2000) [21] labelled a variety of peptides with opioid function that descend from all the casein groups (beta- casein, alpha casein and kappa-casein), whey fraction proteins (beta-lactoglobulin, alpha-lactalbumin) and serum albumin. BCMs are very stable to enzymatic degradation. They act as substrate for dipeptidyl peptidase IV (DPP IV), which is a cell-surface protease belonging to the prolyl oligopeptidase (PO) family. BCM gets hydrolysed by DPP IV to a mixture of Phe-Pro-Gly, Tyr-Pro, Phe-Pro, and Gly (Kreil. 1983) [14]. Dipeptidyl-peptidase IV is expressed in a variety of mammalian cells (Lambeir *et al*., 2003) [16]. Kaminski *et al*., (2007) [6] has found a correlation between serum DPP IV activity of 2 groups of infants (healthy and allergic) and BCM-5 and BCM-7 content of their mother's milk. In the allergic group, the high level of BCM in mother's milk corresponds to the low DPP IV activity in infant's serum. The lower BCM-5 and BCM-7 content of mothers' milk of the allergic group depicts that BCMs can pass from the intestine to the blood and might have prolonged half-life due to a lower DPP IV activity.

Beta-casomorphin-7

BCM-7 (Tyr-Pro-Phe-Pro-Gly-Pro-Ile) was primarily isolated as a peptide possesing morphine-like activity in 1979 (Brantl *et al*., 1979) [2]. This bioactive peptide exhibits a strong opioid activity (Kurek *et al*., 1992) [15] and has been shown to stimulate human lymphocyte T proliferation *in vitro* (Gill *et al*., 2000) [9]. It has also cyto modulatory properties (Meisel and Bockelmann. 1999) [20]. It was observed that *in vitro*, BCM-7 is produced by the successive gastrointestinal proteolytic digestion of beta-casein A1 and B (but not A2) by pepsin, pancreatic elastase, and leucine aminopeptidase (Elliott *et al*., 1999) [7]. Elastase cleaves the peptide bond between Ile and His, releasing the carboxyl terminus of BCM-7. Pepsin and leucine aminopeptidase are essential to release the amino terminus of this peptide (Jinsmaa and Yoshikawa. 1999) [10]. The natural mutation that gave rise to this difference is a result of a single nucleotide polymorphism at codon 67 of the beta-casein gene: CCT (A2, proline), CAT (A1, histidine). This difference in AA sequence suggests a conformational difference in the secondary structure of the expressed protein. It may exert an influence on the physical properties of the respective casein micelles (Elliott *et al*., 1999; McLachlan 2001) [7, 19].

The BCM-7 content of fresh and hydrolysed (digested by pepsin) bovine milk has been examined by Ciecelinska *et al*., 2007 [6] and found that in hydrolysed milk with variant A1 of beta-casein, there is a 4-fold higher level of BCM-7 than in A2 milk (average 11.59 and 2.87 µg mg–1of extract, respectively). In fresh milk, there were little traces of BCM-7.

Beta-casomorphin-7 and human diseases

Ischaemic heart disease

Ischaemic (or coronary) heart disease (IHD or CHD) is one of the major cardiovascular diseases. The A1/A2 theory claims that, more intake of A1 beta-casein would be a risk factor for IHD (McLachlan. 2001) [19]. Epidemiological evidence from New Zealand suggests that A2 milk is better for human health than A1 milk. McLachlan (2001) [19] proved the association between beta-casein A1 consumption and heart disease incidence for 30–69-year-old males across 16 countries (Australia, Austria, Canada, Denmark, Finland, France, Iceland, Israel, Japan, New Zealand, Norway, Scotland, Sweden, United Kingdom, USA, West Germany). He calculated the relationship between the mortality rate from IHD and consumption of milk proteins and milk components. He noted a strong correlation between IHD and A1 consumption. Consumption of beta-casein A1 was also correlated with common risk factors in food (animal fats and red meat) and traditional risk factors (current smokers, hypertensives, body mass index, and serum cholesterol level). Information on these other risk factors (except variant of beta-casein) does not indicate any significant regional difference. Relationships presented by McLachlan (2001) [19] postulates that beta-casein A1, or possibly fragment of the peptide (BCM-7), may be a significant contributor to the etiology of cardiovascular disease. Rabbits fed with beta-casein A1 milk had higher cholesterol levels and higher percent surface area of aorta covered by fatty streaks than those fed with beta-casein A2. On the other hand, A2 beta-casein consumption can protect against IHD, as low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol levels were lesser on the A2 diet than on the A1 diet.

Diabetes mellitus

Diabetes mellitus type 1 (DM-1) incidence has been upsurging globally at 3% per annum (Laugesen and Elliott 2003) [17]. It is an autoimmune disease where the pancreas loses its ability to produce insulin. It develops as a result of the obliteration of the insulin-secreting pancreatic β cells.

Cavallo *et al*., (1996) [4] showed that antibodies against beta-casein increased in DM-1. In addition, epidemiological studies revealed a considerable association between the intake of A1 milk (but not A2 milk) and the incidence of DM-1 (Elliott *et al*., 1999; McLachlan 2001; Laugesen and Elliott 2003) [7, 19, 17]. Elliott *et al*., (1999) [7] compared DM-1 incidence in 0-14 year old children from 10 countries (Australia, Canada, Denmark, Finland, Germany, Iceland, New Zealand, Norway, Sweden and USA– San Diego) with the national annual cow milk protein consumption. The nominated countries had a comprehensive set of data for breed composition and for milk protein polymorphism. He revealed that total protein consumption did not correlate with DM-1 incidence, but consumption of the beta-casein A1 variant did cause DM-1. Relation between beta-casein A1 + B consumption and DM-1 was even higher. He showed that in Iceland, where cows are predominantly A2, there were low incidences of diabetes and heart disease. He noted that the distinctive peptide formed mostly from A1 beta-casein and partly from B beta-casein was BCM-7, and this was a hypothetical risk factor of the disease.

Sudden infant death syndrome (SIDS)

Death of infants between the end of the first month and the first year of life is SIDS (Brooks. 1982) [3]. Sun *et al*., (2003) [24] identified that one factor which is common to all children who develop SIDS is, milk – their only food source. Subsequent absorption from the gastrointestinal tract, BCMs can cross the blood-brain barrier because of the infant's immature central nervous system. It has been reported that BCM immunore activity was found in the brain stem of the human infant. Bell *et al*., (2006) [1] claims that infants may soak up BCM-7 due to an juvenile gastrointestinal tract. BCM-7 can possibly affect various opioid receptors in the immune, nervous and endocrine systems. Transport of BCMs and related peptides out of the central nervous system also has been demonstrated in rats and mice. These results clearly indicate that BCM-7 can cross the blood-brain barrier (Sun *et al*., 2003) [24].

Conclusion

The theory that high consumption of A1 beta-casein upsurges the risk of IHD, DM-1, SIDS, schizophrenia and autism is very interesting for basic as well as application studies. However, some pieces of evidence in relation to human illnesses are not strong enough and should be verified. Therefore it is necessary to continue research into the role of BCM-7 (originating from both raw and processed milk) for human health. *In vivo* experiments are obligatory to confirm the presence of BCM-7 in the blood of animal subjects fed a diet containing milk with the substitute beta-casein genotype. Moreover, it is obligatory to study beta-casein polymorphism collectively with other polymorphic milk proteins, as they all important in the casein micelle structure and overall milk properties and milk products. The genetic platform for such a study has already been established in the form of microarray (Kaminski *et al*., 2005, 2006b; Chessa *et al*., 2007) [11, 12, 5]. Thus, we can methodically try to monitor the frequency of beta-casein alleles in bulls and indirectly in cows. If the hypothesis of objectionable role of A1 beta-casein is established, consumers may wish to reduce or remove this from their diet.

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