A1, A2 BETA CASEIN VARIANTS IN COWS – ITS IMPACT ON MODERN HUMAN HEALTH

Narotam Sharma*
Veena Sharma**
Satish Chandra Nautiyal
Prem Raj Singh
R.S. Kushwaha
Shivani Sailwal
Shayan Ghosh
Ahmer Naushad
R.K Singh

ABSTRACT

Milk from a variety of animal species has been included in the diet not only for infants, but also for human adults. Indian breeds of cows are called Bos Indicus. Common forms of beta-casein in are A1 and A2, while B is less common, and A3 and C are rare. At position 67th of the beta-casein chain, proline in variant A2 is substituted by histidine in variant A1. Milk of ancient breeds of cows is called A2 milk. During digestion, proteins in milk are broken down in to peptides. Most of these peptides get converted into amino acids to be absorbed by the blood stream few got excreted in stools and a quantity of manage to get through the leaks in gut wall in to the blood stream while still in peptide form. A1 milk releases Betacasomorphine 7(BCM7), an opium family substance, associated with number of diseases. In Infants Blood Brain Barrier is formed in human body after 3 to 4 years age associated with Autism, Diabetes type 1 and Sudden Death Syndrome in infants. In Adults BCM7 has been implicated in very high incidence of Ulcerative colitis, CAD, (Heart related diseases) Diabetes, Multiple sclerosis, Mental disorders Parkinson & Schizophrenia.

Key words: Beta casomorphin7, autism, bos Taurus, atherosclerosis

* Molecular Research Laboratory, Department of Biochemistry,Shri Guru Ram Rai Institute of Medical & Health Sciences, Patel Nagar, Dehradun-248001.(U.K.) India.

** Department of Bioscience and Biotechnology, Banasthali University Banasthali–304022, Rajasthan.

Introduction

1.1 Proteins Associated with milk and its significance:

Proteins are a very wide family of organic compounds playing an important functional and structural role in living organisms. The side chains of the standard amino acids have different chemical properties that produce a three-dimensional protein structure with different activities[1]. For the protein functionality the amino acid sequence is therefore of critical importance. The sequence of amino acids in a protein is crucial to the role it plays in a living organism, and each protein is defined by a gene and encoded by a specific genetic code. Globally, public health professionals in particular, but also consumers, food producers and food processors, are becoming increasingly aware of the rapidly expanding body of epidemiological evidence linking the prevalence of diseases, such as cardiovascular disease, obesity, hypertension, diabetes and even cancer associated due to dietary factors[2].

Milk of many species is consumed by humans (e.g. goat, sheep, and buffalo) but bovine milk (Bos Taurus) is economically the most significant. Milk protein consists for a large part (± 90%) of the six main milk proteins a-lactalbumin (a-LA), ß-lactoglobulin (ß-LG), a S2 -casein (a S2-CN), a-CN, κ-CN and ß-CN. The other part of the protein fraction (± 10%) consists of minor proteins like bovine serum albumin, gamma caseins, proteose peptones, immunoglobulins, lactoferrine, lactoperoxidase, and a large number of other proteins that occur in very low concentrations (Farrell et al., 2004). The protein fraction shows even further heterogeneity because of post translational modifications like glycosylation, phosphorylation and disulfide binding, genetic polymorphism and the action of proteolytic enzymes on proteins (Fox and Mcsweeney, 1998). The six main milk proteins are all synthesized in the mammary gland and are products of the corresponding milk protein genes. The amino acid composition of these proteins is depending on the DNA sequence of these genes and their concentration in the milk depends on the level of expression of these genes and /or post-transcriptional control of their mRNA. In milk there are 2 major protein groups: caseins and whey proteins. Caseins accounts for 80% of bovine milk protein, whereas whey proteins constitute about 14%. Bovine milk contains 4 caseins: alpha s1 (CSN1S1, 39–46% of total caseins), alpha s2 (CSN1S2, 8–11%), beta (CSN2, 25–35%), kappa (CSN3, 8–15%). There is also gamma-casein, which is a product of degradation of beta-casein (Ostersen et al. 1997; Miller et al. 1990). Caseins are encoded by members of a multi gene family. The genes encoding 4 caseins are found on bovine chromosome

6. There are 13 genetic variants of beta-casein: A1, A2, A3, A4 B, C, D, E, F, H1, H2, I, G. The most common forms of beta-casein in dairy cattle breeds are A1 and A2, while B is less common, and A3 and C are rare[3]. Casein is a small protein consisting of a total of 209 amino acids. At position 67 of the beta-casein chain, proline in variant A2 is substituted by histidine in variant A1. Both variants A1 and A2 are the most common in the most popular dairy cattle worldwide, i.e. Holstein-Friesian. For example, in β-casein an amino acid substitution occurs at position 67 in different variants.

…-Tyr60-Pro61-Phe62-Pro63-Gly64-Pro65-Ile66-(His67)-… β-casein A1 and B variants

…-Tyr60-Pro61-Phe62-Pro63-Gly64-Pro65-Ile66-(Pro67)-… β-casein A2 variants

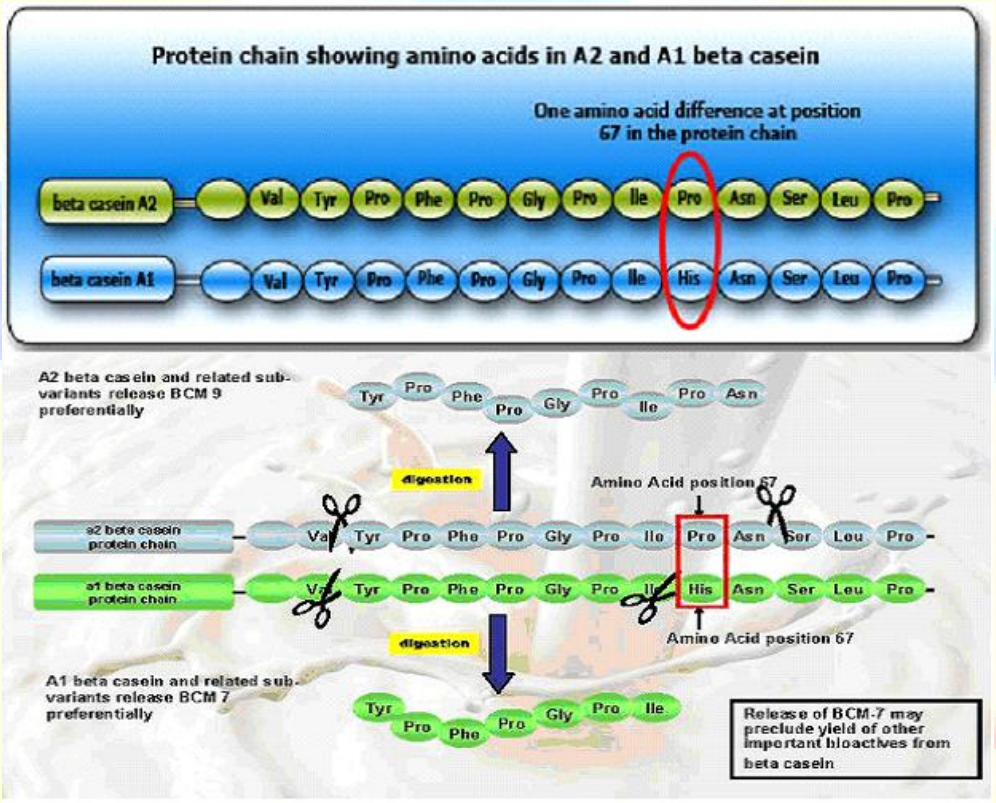

Fig. 1 A1, A2 beta casein variants and release of betacasomorphin 7 from A1 variant.

In β-casein A1 and B variants histidine occurs at position 67, whereas in β-casein A2 proline is present in the same position. This genetic substitution of histidine with proline has been reported to prevent the enzymatic hydrolysis of the peptide bond between residues 66 and 67 in β-casein A2 thereby preventing the release of BCM7. Since genetic polymorphism is breed related, much interest has focused on characterizing β- casein variability in bovine populations, particularly when looking at possible health effects of BCMs[3]. The first evidence of genetic polymorphism in

β-casein came from Aschaffenburg (1961) while studying milk from Jersey and Guernsey cows. By employing paper electrophoresis in the presence of 6.0 M urea in citrate-phosphate buffer pH 7.5, he reported that β-casein existed as three polymorphs, designated in order of decreasing mobility as β-casein A, B and C. Subsequently, Peterson and Kopfler (1966), using acid urea polyacrylamide gel electrophoresis discovered that β-casein A could be separated into three additional variants, now known as β-casein A1, A2 and A3[4]. These variants vary from each other by the number of positively charged His residues in the primary sequence, i.e., 6, 5 and 4, respectively. More recently, the application of chromatographic separation, isoelectric focusing and DNA-based techniques has led to the discovery of a range of other β-casein polymorphs. Numerous studies have been performed on the allele frequency distributions of the three main species of the genus *Bos*, i.e. *Bos taurus* (taurines), *Bos indicus* (zebu) and *Bos grunniens* (yak). However, to date most studies have been performed on taurines as they are the main milk producing cattle breed in the Western globe. The most usually occurring β-casein variants in Western cattle breeds are A1, A2 and B. Country related differences in the β-casein allele frequencies are observed which may be reflective of local breeding policies, some cross-breeding and most prominently targeted breeding for increased milk production traits[5]. At the end of the 1990s, some reports suggested casein variant A1 consumption as a risk factor for type 1 (insulin-dependent) Diabetes Mellitus and Ischaemic Heart Disease in humans[6]. It is known that beta-casein variant A1 yields the bioactive peptide beta-casomorphin-7 (BCM-7), which has been implicated in the development of some human diseases including Autism, Schizophrenia and Sudden Infant Death Syndrome (SIDS).i.e, ß-casomorphin-7 (BCM7), a peptide sequence present in the milk protein ß-casein, has been suggested to contribute to an increased risk for certain non-communicable diseases, such as autism, cardiovascular diseases and type I diabetes[7]. Beta-casomorphins are a group of opioid peptides which can be released from ß-casein[8]. The ß-casein derived peptide with the sequence Tyr60 61-Pro62-Phe63-Pro-Gly6465-Pro-Ile66 is known as ß-casomorphin-7. The release of BCM7 through enzymatic digestion of bovine ß-casein is dictated by different amino acid sequences of this protein. The sequences vary genetically between cow breeds. The amino acid present in position 67 of the sequence in ß-casein appears to be critical for the release of BCM7. In the A2 variant of ß-casein a Proline residue occurs at position 67, where as the A1and B variants of ß-casein have a Histidine residue at this position. In the case of the variants containing Proline the enzymatic hydrolysis of the

Ile66 67-Pro bond does not occur or occurs at a very low rate[9]. The proportion of the different protein variants expressed in the milk, including those of ß casein, is related to their allele distributions in the various cattle breeds and populations. Changing selection targets in the last decades has resulted in changes in bovine breed composition.

Taking into account the lack of detailed knowledge in milk protein variant composition and the diverse geographical origin of dairy products and ingredients across Europe, inadequate information is currently available on the exposure of individual consumers to different ß-casein variants[10]. The first and foremost strategy which can be employed in screening cattle goes to SNP (Single Nucleotide Polymorphism) where every animal's genome specify DNA sequences will vary by virtue of just a single nucleotide , which in turn affects its phenotype. Thus by genotyping a cow, whether it produces a wild type B casein or the mutant type B casein it opens up an opportunity to selectively screen and eliminate the A1 mutant variant cows from the gene pool which in turn leads to production of only A2 B casein variant containing milk. The gene for A1 and A2 beta casein is identifiable by a single nucleotide polymorphism (SNP) [11]. An SNP is a DNA sequence at a location in an animal's genome, which is different to the DNA sequence at the same location in the genome of another animal by virtue of only one nucleotide. Even a difference as small as this can mean an animal exhibits a particular physical trait whereas another animal does not. In addition to phenotyping a cow by identifying the particular β-casein variant or variants produced in the cow's milk, it is well known to genotype a cow by identifying the SNP it possesses to gain knowledge of whether the cow has the ability to produce certain β-casein variant. A method of selecting bovine cows on the basis of such genotyping to form milking herds which will produce milk free of the ß-casein A1 variant, and preferably solely the β-casein A2 variant.

2. Significance of A1, A2 alleles in bovines

It is possible to determine whether a bovine animal possesses a gene for the A1 casein or a gene for the ß-casein A2 protein, not by identifying the DNA of that gene, but by identifying SNPs or haplotypes (combinations of SNPs) in the region of the animal's genome where the gene for β-casein is located. An resourceful assumption was developed by RB Elliott and CNS McLachlan and collaborators in the 1990s, that a protein in the milk of some cows—not others—is an important risk factor for type I diabetes (DM-I) and coronary heart disease (HD) (possibly

also schizophrenia and autism). The implicated protein is the A1 form of b-casein, the second most abundant protein in cow's milk: its commonest genetic variants are A1, A2 and B b-casein [12]. Elliott (1992) was struck by the very low incidence of DM-I among children in Polynesian islands like W. Samoa, compared with Polynesian children in Auckland[13]. Prolonged breast feeding appears to be protective against DM-I. Cow's milk antibodies are found at higher levels in diabetic children than in controls. DM-I rates between countries correspond fairly well with cow's milk intake. The next step was animal experiments with a strain of mice genetically susceptible to diabetes, the non-obese diabetic (NOD) mouse[14]. When fed for 250 days from weaning on diets containing A1 b-casein nearly half became diabetic but no diabetes occurred in the mice fed A2 b-casein[15]. Meanwhile CNS McLachlan, also in Auckland, New Zealand produced evidence for a correlation of mortality from CHD in 16 countries with national A1 b-casein consumption (g / day). McLachlan omitted A1 b-casein in cheese and b-casein type B[16]. The numbers he calculated for national A1 b-casein consumption were not the same as in Elliott's correlations. He chose 5 y as the lag phase between food intake and CHD mortality. McLachlan's evidence was discussed within the dairy industry and in 1996 he applied for patents in New Zealand (McLachlan, 1996a) and with the WTO (McLachlan, 1996b), contending that consumption of a specific common variant of the milk protein b-casein (b-casein A1) promotes the development of heart disease in humans[17]. His data were not published in the scientific literature until 2001 in Medical Hypotheses (McLachlan, 2001). To add to this ecological data, Tailford et al (2003) reported a rabbit experiment in which rabbits killed after feeding for 6 weeks with 10% A1 b-casein showed larger areas of aortic fatty streaks than animals that received A2 b-casein.

3. The Scientific Evidence:

There are at least eight strands to the evidence, with more than 100 relevant papers in the peer reviewed medical and science literature. The first strand is remarkable epidemiological evidence that countries with high intakes of A1 beta-casein are the same countries that have high levels of Type 1 diabetes (Fig. 2) and heart disease (Fig. 3).

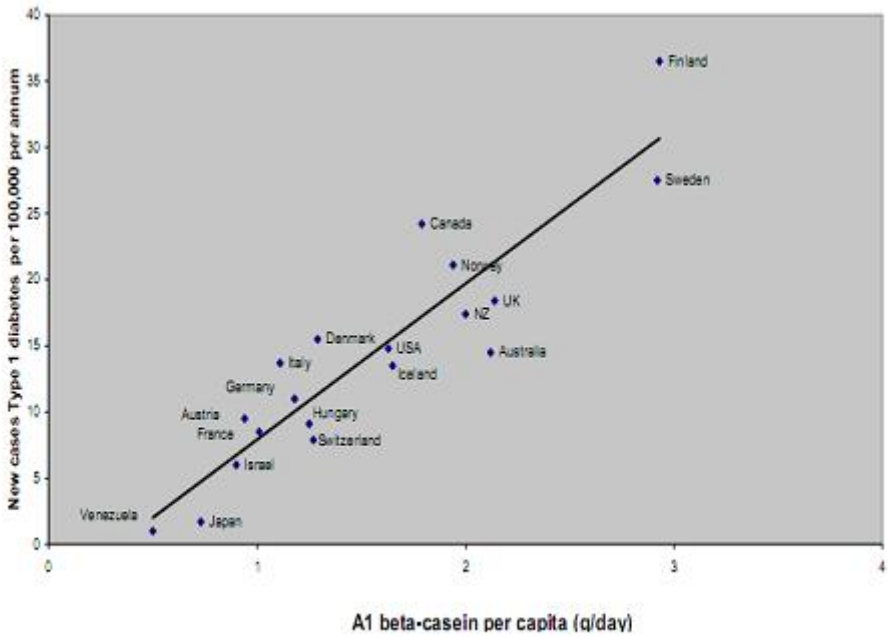

Fig. 2 Incidence of Type 1diabetes and A1 beta casein intake.

Type 1 diabetes is the form that typically develops in childhood and requires insulin injections. The statistical associations are extremely strong such that it is highly unlikely to be due to random factors. In terms of alternative possibilities that have been put forward there are none that explain the statistical association. The second strand is the biochemical knowledge that A1 and A2 beta-casein digest differently. Empirical evidence from at least three laboratories confirms that, *in vitro* and in the presence of digestive enzymes, A1 beta-casein releases large amounts of beta-casomorphin7 (BCM7) whereas A2 milk does not (Hartwig *et al* 1997, Jinsmaa and Yoshikawa 1999).

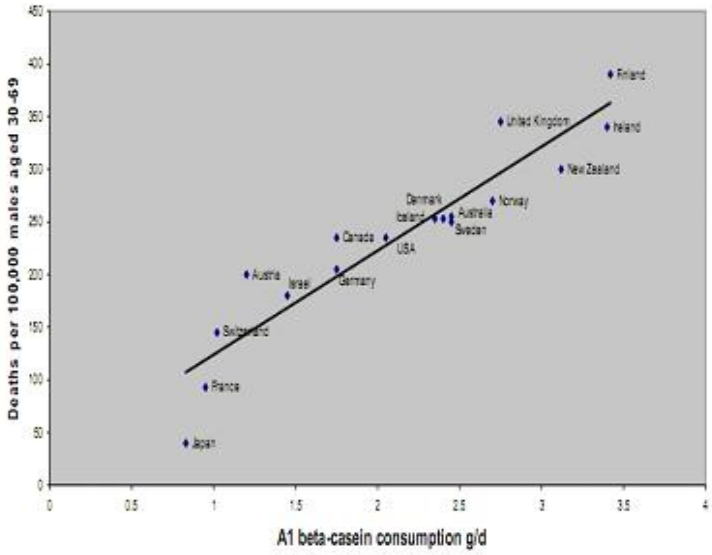

Fig.3. A1 beta- casein consumption excluding cheese and deaths from ischaemic heart disease

The third strand of evidence is that BCM7 is known with certainty to be a powerful opioid. This has been known for many years from laboratory work (Brantl and Teschemacher 1994). The effects have also been clearly demonstrated when BCM7 is injected into rats (Sun *et al* 1999). The effects can be counteracted by the use of naloxone which is an opioid antagonist.

4. Some Basic Genetics:

The A1/A2 status of a cow is determined by a pair of genes on the sixth chromosome. There are two major alleles (or variants) of the gene. These are called the A1 and A2 beta-casein alleles. Because a cow carries two copies of the beta-casein gene, she can carry either two copies of the A2 allele, or one copy of each of the A1 and A2 alleles, or two copies of the A1 allele. The three states are referred to as being homozygous A2A2, heterozygous A1A2, or homozygous A1A1. Neither allele is dominant over the other. Instead they are co-dominant, i.e. additive in their effect. Therefore an A1A2 cow will produce A1 and A2 beta-casein in equal amounts. An A2A2 cow will only produce A2 beta-casein and an A1A1 cow will only produce A1 beta-casein. As a very rough generalization, herds based on the Northern European black and white breeds such as the Friesian Holstein typically carry the A1 and A2 allele at about equal levels. The Southern European breeds and the Jersey are likely to carry the A1 allele at about 35%. There are plenty of exceptions. For example the Guernsey breed appears to carry the A1 allele at less than 10% and the Scottish Ayrshire breed appears to be well over 50%. In addition, individual herds may carry the allele at levels that are quite different to the average for the breed.

If a cow is A2A2 then she is guaranteed to pass on the A2 allele to her progeny. Similarly, an A1 cow is guaranteed to pass on the A1 allele. For an A1A2 cow there is a 50% chance of passing on either allele. Breeding for A2A2 cows is based on using semen for bulls that have been tested as being A2A2. The breeding process can be speeded up by selective culling of A1A1 and A1A2 cows and by selective retention of A2A2 calves. The speed at which a herd will be converted to A2 milk production depends on whether the strategy relies solely on use of A2 semen (the passive approach) or a more active strategy that requires testing all cows together with selective culling and calf retention. Because a cow carries two copies of the beta-casein gene, she can carry either two copies of the A2 allele, or one copy of each of the A1 and A2 alleles, or two copies of the A1 allele. The three states are referred to as being homozygous A2A2, heterozygous A1A2, or homozygous A1A1.

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If farmers rely on the passive approach, and assuming that cows start milk production at two years of age, it will take 2.75 years subsequent to mating decisions before there is any impact in the milk vat. Thereafter, and assuming an initial A1:A2 ratio of 50:50, then the A2 proportion will increase each year by about 5 percentage units (e.g. to 55:45 once the first cohort of specially bred heifers enters the milking herd approximately three years after conception). However, the rate of improvement gradually slows down (the relationship is asymptotic) and a herd will never reach 100% A2 without testing of cows. For farmers who start with A2 semen and then complete the process by testing cows and selecting only A2A2 replacements, the total process is likely to take about two cow generations, i.e. about 10 years.

5. Potential health impact of β-casomorphins and related peptides on modern human health:

5.1 Heart disease.

As with Type 1 diabetes, major differences in incidence between countries within the developed world correlate remarkably with A1 beta-casein intake. In babies, formula-fed babies have been shown to have high antibodies to oxidized LDL. In piglets, a direct trial comparison has shown a statistically significant 'cause and effect' relationship between A1 beta-casein intake and oxidized LDL antibodies. In rabbits, a 'cause and effect'

relationship has been shown with A1 beta-casein leading to a statistically significant build-up of arterial plaque (19). In humans, it has long been recognized that high milk diets *Milk Proteins and Human Health* GPCE Sydney 2011 Keith Woodford 3 for stomach ulcer sufferers (a potential cause of stomach permeability) lead to high death rates from heart disease[18].

5.2 Child development

Russian scientists have shown that BCM7 enters the blood of babies fed milk formula diets.Whereas some babies can quickly metabolize the BCM7, others are slow metabolisers. In babies whose BCM7 levels in the blood stay high between feeds, there is a high risk of delayed psychomotor development[19].

5.3 Sudden Infant Death Syndrome (SIDS)

BCM7 has been suspected as a risk factor for SIDS for more than 20 years. Casomorphins have been found in the brainstems of children who have died from SIDS but comparisons with normal children are obviously not possible. Until recently, direct evidence of apnoea-inducing effects was only available from animals. However, specific evidence of BCM7 causing respiratory depression in humans has now come from Polish scientists who have shown that babies who suffer acute life threatening events (ALTE) through apnoea are characterized by circulating levels of BCM7 that are three times higher than in normal children . These same children have DPP4 levels (the enzyme that degrades BCM7) that are only 58±3% of those in normal children. The evidence is that even if the babies are breast-fed, bovine BCM7 is still found in the blood of the infants. This suggests bovine BCM7 is transferring from the mother's stomach to her infant via human milk. Other work by this group has found bovine BCM5 (a stronger opioid which is derived from BCM7) in the blood of breast-fed children[20].

5.4 Autism

Autism is best viewed as a spectrum of conditions. BCM7 has long been considered a risk factor for autism but the hypothesis remains controversial. Trials with animals show that BCM7 crosses the blood-brain barrier and leads to autistic type behavior . Milk elimination trials in humans have produced positive results but are often criticised for lack of double blind protocols. Many autistic children suffer from digestive complaints Milk Proteins and Human *Health* GPCE Sydney 2011 Keith Woodford 4 which may make them susceptible to BCM7 absorption . There are theoretical grounds to suspect that BCM7 reaching the brain will affect the seratoninergic system with implications for neurological development[21].

5.5 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 BCM7 that is released from A1 beta-casein slows down the passage of food through the digestive system (as do other opioids) providing longer time for lactose fermentation (Given that

fermentation is an exponential process, a modest slowing down can lead to major production of gas and other fermentation products.) Second, many people are intolerant specifically to the BCM7. 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 my experience is that they can also tolerate A2 milk.

5.6 Mild allergies

In theory, an allergy is quite distinct from intolerance. 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 BCM7 can include eczema and asthma, with much of the evidence being case-related. BCM7 is also known to induce production mucins (the sticky proteins in mucus) (30-33) 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 BCM7 which can cause severe reactions including anaphylactic shock in susceptible people[22-25].

5.7 Type 1 diabetes

Evidence connecting A1 beta casein to Type 1 (insulin-dependent) diabetes includes major differences in incidence between individual countries within the developed world (i.e. countries with similar lifestyles). These differences correlate remarkably with A1 beta-casein intake of these populations. Alternative hypotheses are unable to explain this. Supportive evidence comes from animal trials, evidence linking IDDM to milk exposure in general and A1 beta-casein in particular[26]. The increasing incidence of Type 1 diabetes over time is likely to be a function of issues affecting gut permeability or antigenic susceptibility (viruses, antibiotics, hygiene factor, Vitamin D etc.) rather than the quantity of A1 beta-casein. (Data from Laugesen and Elliott).

Conclusion:

Native Indian breeds cows Bos Indicus, represent the world's major potential source of 'good' A2 type BCM7 free (Beta Casomorphine 7) milk for the world. While in short term dairy experts the world over may ostensibly choose to ignore or question the New Zealand research hypothesis on quality of milk, but it can not be denied that there is overwhelming medical evidence weighted in flavor of BCM7 free A2 milk. India must also be aware that quietly every Dairy nation is planning a change in its herd breeding policies. Within next few years India will be abruptly coming with a very large A2 milk production all over the world.

If the dairy industry so wished, it could breed out A1 beta-casein from the national herd over a period of 10 years, and do so at minimal cost. In the meantime, milk free of A1 beta-casein (i.e. 'a2 Milk™' and goat milk) is widely available. Some infant formulas that are free of all casein are available as specialist products, but an A2 infant formula is not yet available. Neither is it currently possible to purchase either ice cream or cheese made from A2 milk.

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