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Review article

Polymorphism of bovine beta-casein and its potential effect on human health

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Abstract. Proteins in bovine milk are a common source of bioactive peptides. The peptides are released by the digestion of caseins and whey proteins. In vitro the bioactive peptide beta-casomorphin 7 (BCM-7) is yielded by the successive gastrointestinal proteolytic digestion of bovine beta-casein variants A1 and B, but this was not seen in variant A2. In hydrolysed milk with variant A1 of beta-casein, BCM-7 level is 4-fold higher than in A2 milk. Variants A1 and A2 of beta-casein are common among many dairy cattle breeds. A1 is the most frequent in Holstein-Friesian (0.310–0.660), Ayrshire (0.432–0.720) and Red (0.710) cattle. In contrast, a high frequency of A2 is observed in Guernsey (0.880–0.970) and Jersey (0.490–0.721) cattle. BCM-7 may play a role in the aetiology of human diseases. Epidemiological evidence from New Zealand claims that consumption of beta-casein A1 is associated with higher national mortality rates from ischaemic heart disease. It seems that the populations that consume milk containing high levels of beta-casein A2 have a lower incidence of cardiovascular disease and type 1 diabetes. BCM-7 has also been suggested as a possible cause of sudden infant death syndrome. In addition, neurological disorders, such as autism and schizophrenia, seem to be associated with milk consumption and a higher level of BCM-7. Therefore, careful attention should be paid to that protein polymorphism, and deeper research is needed to verify the range and nature of its interactions with the human gastrointestinal tract and whole organism.

Keywords: beta-casein, beta-casomorphin 7, diabetes, ischaemic heart disease, polymorphism.

Introduction

Milk is the most important food for young mammals and a common source of proteins and microelements for adult people. In milk there are 2 major protein groups: caseins and whey proteins. Caseins account for ca. 80% of bovine milk protein (Niki et al.1994; Martien et al. 1994), whereas both major whey proteins constitute about 14% (McLachlan 2001; Roginski 2003).

Bovine milk contains 4 caseins: alpha s1 (CSN1S1, 39–46% of total caseins), alpha s2 (CSN1S2, 8–11%), beta (CSN2, 25–35%), and kappa (CSN3, 8–15%) (Eigel et al. 1984; Roginski 2003). There is also gamma-casein, which is a product of degradation of beta-casein (Ostersen et al. 1997; Miller et al. 1990). Whey protein is composed of beta-lactoglobulin (LGB), alpha-lactoalbumin (LALBA), immunoglobins (IgGs), glycomacropeptides (GMP), bovine serum albumin (BSA), and minor proteins, such as lactoperoxidase, lysozyme and lactoferrin (Farrell et al. 2004).

Caseins are encoded by members of a multigene family. The genes encoding 4 caseins are found on bovine chromosome 6 (Rijnkels 2002). This study is focused on one of them, the beta-casein gene, and discusses the potential influence of beta-casein variants on human health.

Beta-casein polymorphism

There are 13 genetic variants of beta-casein: A1, A2, A3, B, C, D, E, F, H1, H2, I, G (Table 1). For the A4 allele, found in Korean native cattle,

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Table 1. Changes in the amino acid sequence of beta-casein variants

Beta-casein variants	Change in amino acid sequence													
	18	25	35	36	37	67	72	88	93	106	117	122	137	138
A2	Ser-P	Arg	Ser-P	Glu	Glu	Pro	Glu	Leu	Gln	His	Gln	Ser	Leu	Pro
A1						His								
A3										Gln				
B						His						Arg		
C			Ser		Lys	His								
D	Lys													
E				Lys										
F						His								Leu
G						His						Leu		
H1		Cys						Ile						
H2							Glu		Leu					Glu
I									Leu					

nucleotide substitution is not yet recognized. 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 (Farrell et al. 2004). In position 67 of the beta-casein chain, proline in variant A2 is substituted by histidine in variant A1 (Groves 1969, Roginski 2003). Both variants – A1 and A2 – are the most common in the most popular dairy cattle worldwide, i.e. Holstein-Friesian.

Milk proteins as a source of active peptides

Some milk proteins are the source of active peptides – opioids (Brantl et al. 1979; Chang et al. 1985; Kostyra et al. 2004). The term 'opioid' refers to chemical substances that have a morphine-like activity in the body. Some of them are known to play an important role in the response to stress and pain, and the control of food intake. These agents act by binding to opioid μ-receptors, which are found principally in the central nervous system and the gastrointestinal tract (Teschemacher 2003).

They are inactive within the sequence of the parent protein and can be released during gastrointestinal digestion (hydrolysis in the stomach by digestive enzymes) or food processing (digestion or maturation during technological processing), or produced by the body itself. Proteolysis during milk fermentation and cheese ripening (by using microbial enzymes) also leads to the formation of various bioactive peptides (Korhonen et al. 1998; Hartwig et al. 1997; Meisel 1997; Jinsmaa and Yoshikawa 1999; Gobbetti et al. 2002; Kostyra et al. 2002).

At the end of the 1990s, some reports suggested casein variant A1 consumption as a risk factor of type 1 (insulin-dependent) diabetes mellitus (Elliott et al. 1999) and ischaemic heart disease in humans (McLachlan 2001). It is thought that beta-casein variant A1 yields the bioactive peptide beta-casomorphin-7, which may play an unclear role in the development of some human diseases. Also, a relation of beta-casomorphin to sudden infant death syndrome (SIDS) has been suggested (Sun et al. 2003). Another potential impact of milk proteins on human health is its hypothetical correlation with milk allergy (Chatchatee et al. 2001a, 2001b; Gobbetti et al. 2002).

Frequency of beta-casein A1 and A2 alleles in various dairy cattle breeds

The frequency of the *CSN2* alleles in various dairy breeds and countries is shown in Table 2.

Many data based on starch gel electrophoresis allowing for differentiating only A, B and C alleles were excluded from the current comparison of *CSN2* allele A1/A2 frequency.

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). The group of these peptides isolated from bovine and human beta-casein (Ramabadran and Bansinath 1989) is shown in Table 3.

Meisel and Fitzgerald (2000) described a range of peptides with opioid function that derive from all the casein groups (alpha-casein, beta- casein, kappa-casein), whey fraction proteins (alpha-lactalbumin, beta-lactoglobulin) and serum albumin.

BCMs are very stable with regard to enzymatic degradation. They are a substrate for dipeptidyl-

Table 2. Occurrence of beta-casein gene variants in various breeds and countries (data sorted by increasing A1 allele frequency)

Breed	Country	No. of animals	Frequency of beta-casein alleles			References
			B	A1	A2	
Guernsey	USA	400		0.010		Swaissgood 1992
	USA	3861	0.010–0.020	0.010–0.060	0.880–0.970	Enennam et al. 1991
Jersey	Germany	43	0.186	0.093	0.721	Ehrmann et al. 1997
	Denmark	157	0.350	0.070	0.580–0.650	Bech et al. 1990
	New Zealand	1328	–	0.123	0.591	Winkelman and Wickham 1997
	USA	387	0.290–0.370	0.090–0.220	0.490–0.540	Eenennam et al. 1991
Brown Swedish	Germany	232	0.170	0.108	0.705	Ehrmann et al. 1997
	USA	282	0.100–0.180	0.140–0.150	0.660–0.720	Swaissgood 1992
	USA	259	0.100–0.180	0.140–0.180	0.660–0.720	Eenennam et al. 1991
Simmental	Croatia	621	0.150	0.190	0.630	Curik et al. 1997
	Germany	229	–	0.343	0.566	Ehrmann et al. 1997
HF	USA	526	0.010–0.060	0.310–0.660	0.240–0.620	Swaissgood 1992
	USA	6000	0.010–0.040	0.310–0.490	0.490–0.620	Eenennam et al. 1991
	Hungary	768	0.107	0.418	0.470	Baranyi et al. 1997
	Germany	229	0.026	0.472	0.496	Ehrmann et al. 1997
	Poland	143	–	0.402	0.598	Kamiński et al. 2006a
	New Zealand	3761	–	0.465	0.510	Winkelman et al. 1997
	Norway	306	0.010	0.400	0.490	Lien et al. 1993
Black-and-White	Denmark	223	0.030–0.080	0.550	0.390	Bech et al. 1990
Red-and-White	Sweden	394	0.008	0.460	0.531	Lunden et al. 1997
	Germany	179	0.020	0.573	0.366	Ehrmann et al. 1997
Ayrshire	New Zealand	37	–	0.432	0.527	Winkelman and Wickham 1997
	Finland	686	0.001	0.509	0.490	Ikonen 1997
	United Kingdom	29	0–0.003	0.600	0.400	Swaissgood 1992
	USA	45	0	0.720	0.280	Swaissgood 1992
Red	Denmark	169	0.044–0.060	0.710	0.230	Bech et al. 1990

Table 3. Amino acid composition of naturally occurring beta-casomorphins (BCMs) in human and bovine milk

Beta-casomorphin	Amino acid composition
Bovine BCM-4	Tyr-Pro-Phe-Pro
Bovine BCM-5	Tyr-Pro-Phe-Pro-Gly
Bovine BCM-6	Tyr-Pro-Phe-Pro-Gly-Pro
Bovine BCM-7	Tyr-Pro-Phe-Pro-Gly-Pro-Ile
Bovine BCM-8	Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro
Bovine BCM-11	Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-Ser-Leu
Human BCM-7	Tyr-Pro-Phe-Val-Glu-Pro-Ile
Human BCM-8	Tyr-Pro-Phe-Val-Glu-Pro-Ile-Pro

peptidase IV (DPP IV), which is a cell-surface protease belonging to the prolyl oligopeptidase (PO) family. BCM is hydrolysed by DPP IV to a mixture of Tyr-Pro, Phe-Pro-Gly, Phe-Pro, and Gly (Kreil 1983).

Dipeptidyl-peptidase IV is expressed in a variety of mammalian cells (Lambeir et al. 2003). Jarmołowska et al. (2007) 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 mothers' milk. In the allergic group, the high level of BCM in mother's milk corresponds to the low DPP IV activity in infant's sera. The lower BCM-5 and BCM-7 content of mothers' milk of the allergic group seem that BCMs can pass from the intestine to the blood and may have prolonged half-life due to a lower DPP IV activity.

Beta-casomorphin 7

BCM-7 (Tyr-Pro-Phe-Pro-Gly-Pro-Ile) was first isolated as a peptide having morphine-like activity in 1979 (Brantl et al. 1979). This bioactive peptide exhibits a strong opioid activity (Kurek et al. 1992) and has been shown to stimulate human lymphocyte T proliferation in vitro (Gill et al. 2000). It has also cytomodulatory properties (Meisel and Bockelmann 1999).

The sequence of BCM-7 corresponds to positions 60-66 of the bovine beta-casein aa sequence. Human BCM-7 (positions 51–57 of human beta-casein) is similar to the bovine one. Both peptides have 7 aa with the sequence Tyr-Pro-Phe- at the N-terminus and both have opiate properties within or close to the BCM-7 sequence. The basic difference is that in human beta-casein, polymorphism was not observed (A2 Corporation 2006).

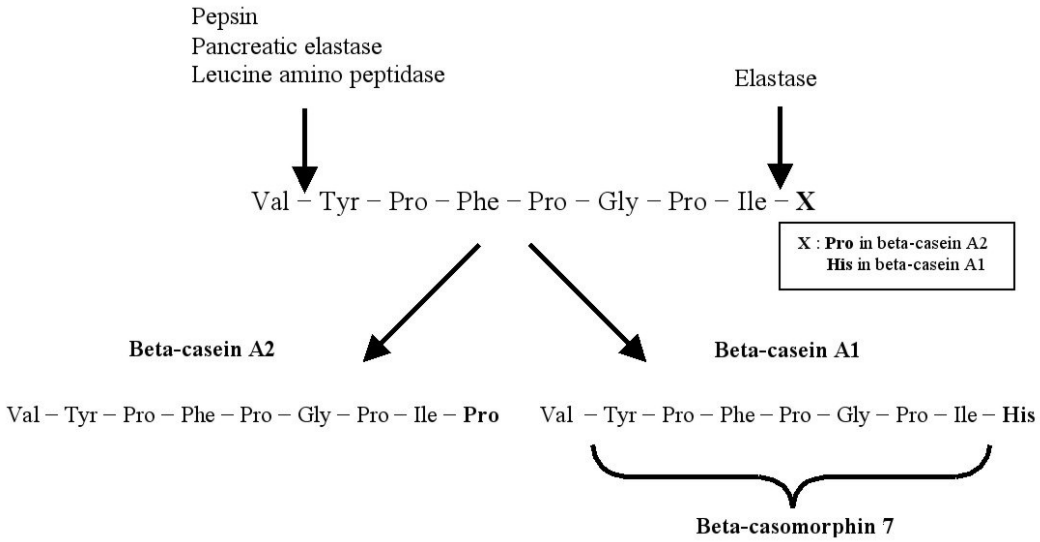

Figure 1. Release of beta-casomorphin-7 from beta-casein variant A1 but not from A2

It was shown (Hartwig 1997, Jinsmaa and Yoshikawa 1999; Cieślińska et al. 2007) that in vitro the bioactive peptide BCM-7 is yielded 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). Elastase cleaves the peptide bond between Ile and His, releasing the carboxyl terminus of BCM-7. Pepsin and leucine aminopeptidase are required to release the amino terminus of this peptide (Jinsmaa and Yoshikawa 1999) (Figure 1). 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 1999; McLachlan 2001).

The BCM-7 content of fresh and hydrolysed (digested by pepsin) bovine milk has been examined by our group lately. We have 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^{-1} of extract, respectively). In fresh milk, there were traces of BCM-7 (Cieślińska et al. 2007).

Precursors of BCMs were found in Swiss, Blue, Cheddar, Limburger and Brie cheeses. However, BCMs were not found because they could have been degraded by proteolytic enzymes from the starter culture, or these peptides might be present in undetectable amounts (Muehlenkamp and Warthesen 1996). Only Jarmołowska et al. (1999) found BCM-7 in Kaszkawal, Camping and Brie cheeses.

Sturner and Chang (1988) identified large quantities of BCM-like and morphiceptin-like activities in infant formulas. These findings are confirmed by current observations (Kostyra E., pers. comm.).

Beta-casomorphin 7 and human diseases

Ischaemic heart disease

It has been shown that bovine BCM-7 may be a risk factor for human ischaemic heart disease, atherosclerosis, type 1 diabetes, and sudden infant death syndrome (Elliott et al. 1999; Thorsdottir et al. 2000; McLachlan 2001; Laugesen and Elliott 2003; Sun et al. 2003; Tailford et al. 2003).

Correlations between A1 beta-casein consumption and human disease were based on epidemiological studies. The risk factor data for several diseases and mortality data were taken from the World Health Organization (WHO). Nutritional data and dairy protein consumption data were based on data from the Food and Agriculture Organization (FAO), food supply data and

beta-casein fractions were estimated by breed from the dairy science literature.

Ischaemic (or coronary) heart disease (IHD or CHD) is one of the major cardiovascular diseases. The A1/A2 hypothesis claims that a high intake of A1 beta-casein is a risk factor for IHD (McLachlan 2001).

Epidemiological evidence from New Zealand suggests that A2 milk is better for human health than A1 milk. McLachlan (2001) showed 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 (r=0.86). 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) postulate that beta-casein A1, or possibly fragment of the peptide (BCM-7), may be a significant contributor to the aetiology of cardiovascular disease.

Also, Laugesen and Elliott (2003) in epidemiological studies showed a relationship between A1 beta-casein and IHD mortality in 35–64-year-old males across 19 countries (Australia, Austria, Canada, Denmark, Finland, France, Germany, Hungary, Iceland, Israel, Italy, Japan, New Zealand, Norway, Sweden, Switzerland, United Kingdom, USA, Venezuela). A1/capita was estimated as the product of per capita cow milk and cream supply and its A1 beta-casein content (calculated from herd tests and breed distribution, or from tests of commercial milk):

A1/capita = (cow% × (milk protein supply/capita) × (beta-casein/cow milk protein) × (national A1/beta-casein),

where "cow%" is total milk production percentage minus the percentage from sheep and goat milk, and then tested for correlation with IHD in 20 countries. For comparison, they also correlated 77 food and 110 nutritive supply FAO-based measures, against IHD mortality. For IHD, cow milk proteins (A1/capita, r=0.76; milk protein r=0.60) had stronger positive correlations with IHD than fat supply variables. Table 4 shows a simple correlation between consumption of beta-casein variant A1 and other risk factors (tobacco, alcohol and saturated fats) and IHD mortality in selected countries.

Table 4. Comparison of beta-casein variant A1 consumption per capita, other risk factors, and IHD mortality in selected countries (data from Laugesen and Elliott 2003)

Country	A1/capita (g/day)	IHD mortality per 100 000	Other risk factors: Tobacco/Alcohol/Saturated fat (g/day)
Ireland	3.84	131.1	2279/16.2/14.5
Finland	3.11	113.0	1933/16.4/14.2
Iceland	1.82	72.5	2255/8.0/16.6
Italy	1.19	50.5	1907/17.6/12.7
France	0.93	32.8	2204/22.9/16.2

Casein consumption has been found to cause hypercholesterolaemia, or atherosclerosis, in numerous animal studies including rabbits, pigs, monkeys, and rodents (McLachlan 2001). Tailford et al. (2003) fed A1 milk and A2 milk to rabbits. 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 lower on the A2 diet than on the A1 diet.

The physiological effect of BCM-7 in A1 beta-casein on the oxidation of LDL or peroxidation of a lipid component of LDL, regarded as a determining step in the development of heart disease, has been shown (Elliott et al. 1999). Analysis of protein oxidation products isolated from atherosclerotic lesions implicates, e.g., the tyrosyl radical (Heinecke 1999), and BCM-7 is its potential source.

According to these reports, consumption of beta-casein A1 is associated with national mortality rates from IHD.

Diabetes mellitus

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

The hypothesis that casein may play a role in that disease was presented by Cavallo et al.

(1996), who showed that antibodies against beta-casein increased in DM-1. Also, epidemiological studies showed a significant 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).

Elliott et al. (1999) 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 selected countries had a complete set of data for breed composition and for milk protein polymorphism. He showed that total protein consumption did not correlate with DM-1 incidence ($r = 0.402$), but consumption of the beta-casein A1 variant did ($r = 0.726$). Relation between beta-casein A1 + B consumption and DM-1 was even higher ($r = 0.982$). He showed that in Iceland, where cows are predominantly A2, there are low numbers of cases 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.

Similar data in Iceland were presented by Thorsdottir et al. (2000) and Birgisdottir et al. (2006) But they also showed that in Finland, Norway, Denmark and Sweden (similar conditions as in Iceland, except milk protein variants), where the cows are mainly A1, the incidence of the disease is very high.

McLachlan (2001) showed that the consumption of A1 beta-casein across 16 countries is correlated strongly ($r = 0.75$) with the incidence of DM-1 in children under 15 years old.

In 2003, Laugesen and Elliott confirmed the same relationship between A1 and DM-1 across 19 countries in 0–14-year-old children. Correlations were not significant for variant A2 of beta-casein.

In an animal model, fraction A1 of beta-casein has been found to be diabetogenic for non-obese diabetic (NOD) mouse, whereas fraction A2 has not (Elliott et al. 1997).

Several mechanisms have been suggested to explain the risk of DM-1 and milk consumption but all are based on BCM-7. It is released from beta-casein A1 and has been shown to inhibit human intestinal lymphocyte proliferation in vitro. It is possible that such an immune suppressant influences the development of gut-associated immune tolerance, or suppresses defence mechanisms towards enteroviruses, both of which have been implicated in the aetiology of DM-1 (Elliott et al. 1999; Laugesen and Elliott 2003).

The A2 Corporation (2006) explains that A1 beta-casein is cleaved enzymatically in the gut to produce BCM-7, whose morphine-like activity may influence the immune surveillance or response to antigens, such as enteroviruses or endogenous retroviruses, which then damage the pancreatic β-cells.

Sudden Infant Death Syndrome (SIDS)

SIDS is the cause of death of infants between the end of the first month and the first year of life (Brooks 1982). Sun et al. (2003) indicates that one factor which is common to all children who develop SIDS is milk – their only food source. Following absorption from the gastrointestinal tract, BCMs can cross the blood-brain barrier because of the infant's immature central nervous system. In infants with abnormal respiratory control and vagal nerve development, the opioid peptides derived from milk might induce depression of the brain-stem respiratory centres, leading to death. It has been reported that BCM immunoreactivity was found in the brain stem of the human infant. Bell et al. (2006) claims that infants may absorb BCM-7 due to an immature gastrointestinal tract. BCM-7 can potentially affect numerous opioid receptors in the nervous, endocrine, and immune 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).

Other illnesses

Epidemiological studies have also linked the consumption of A1 beta-casein with some neurological disorders, such as autism and schizophrenia. The level of BCM-7 is elevated significantly in urine (Cade et al. 2000; Reichelt et al. 1991) and blood (Lindstrome et al. 1984; Reichelt et al. 1990) of patients with schizophrenia, autism, and postpartum psychosis. To a significant degree, this bioactive peptide crosses gastrointestinal tract mucosa and enters blood in certain individuals. These compounds enter the circulation, cross the blood- brain barrier, and influence neurological functioning (Sun et al. 2003).

Critics of the A1/A2 hypothesis

There are only 2 papers reporting an opposite opinion on BCM-7 role in human health. Especially scientists of Fonterra group (one of the world's largest dairy corporations that control

95% of New Zealand's milk supply) criticize the A1/A2 hypothesis. Fonterra's scientists have been critical about the A1/A2 hypothesis and stated that no convincing relationship has been established between A1 or A2 milk and diseases: heart disease, diabetes, autism, and schizophrenia. They have argued that the data on A1 composition of the herds in various countries is quite limited and potentially unreliable. McLachlan's (2001) data are based on the WHO MONICA (World Health Organisation MONitoring in Cardiovascular Disease Surveys) project, which showed a limited relationship with the CHD mortality rates between countries, and in mirroring the historical rise and decline in deaths from this disease.

Those scientists have disputed that McLachlan's work was flawed because he excluded cheese intake from his analyses. But cheese is made from the same milk, so A1/A2 variants are represented in cheese to the same extent as in raw milk or yoghurt. The question is if cheese is a source of BCM-7. There are 3 reports confirming this hypothesis (Muehlenkamp and Warthensen 1996; Jarmołowska et al. 1999; Norris et al. 2003).

Another Fonterra's argument is based on the fact that they have been able to demonstrate the presence of very small amounts of BCM-7 in human milk. They suggest that undesirable effects may originate not only from bovine milk but also from feeding women's milk. In some cases, it could happen when the physiological level of BCM-7 is exceeded and the activity of DPP IV is low.

Also a review by Truswell (2005) suspends the hypothesis that variant A1 of beta-casein could facilitate the immunological processes that lead to type 1 diabetes. He claims that for both DM-I and CHD, the between-country correlation method is shown to be unreliable and negated by recalculation with more countries and by prospective studies in individuals. In his opinion the number of countries included is too small. He also points out that the animal experiments with diabetes-prone rodents that supported the hypothesis about diabetes were not confirmed by larger, better- standardised multicentre experiments and that the single animal experiment supporting an A1 beta-casein and CHD link was small, short, in an unsuitable animal model and had other design weaknesses.

Attempts to commercialise beta-casein polymorphism

A2 Corporation Ltd. was established in New Zealand in 2000 to test cows and market milk with only the A2 variant of beta-casein. The company has developed a DNA test kit in which a hair is plucked from a cow's tail and then analysed to determine whether the animal is A1 or A2 or a combination of both. The easiest way to use the desirable beta-casein A2 genotypes is selective distribution of bull semen. It will allow to develop herds of cows producing milk with A2 variant only.

Since 2003, A2 milk has been sold in New Zealand and Australia as a premium brand, offering a natural choice in protein content. The company has started marketing a2 milk in Asia and the United States, too (A2 Corporation 2006).

Conclusions and perspectives

The hypothesis that a high consumption of A1 beta-casein increases the risk of DM-1, IHD, SIDS, schizophrenia and autism is very intriguing and 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, e.g. cheese) for human health. In vivo experiments are necessary to verify the presence of BCM-7 in the blood of animal models fed a diet containing milk with the alternative beta-casein genotype. It is also desirable to determine the physiological level of casomorphins in blood and the variance of DPP IV activity in humans. Moreover, it is necessary to study beta-casein polymorphism together with other polymorphic milk proteins, as they all influence the casein micelle structure and overall milk properties and milk products (yoghurt, cheese).

The genetic platform for such a study has already been established in the form of microarray (Kamiński et al. 2005, 2006b; Chessa et al. 2007). Over 4 years ago we already initiated systematic genotyping of Polish Friesian-Holstein bulls for beta-casein. In this way, we systematically try to monitor the frequency of beta-casein alleles in bulls and indirectly in cows.

If the hypothesis of undesirable role of A1 beta-casein is confirmed, consumers may wish to reduce or remove this allele from their diet. This may play an important role for people with established familial IHD or a high risk of IHD, children at high risk for DM-1, and other high-risk families.

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