Milk A1 and A2 Peptides and Diabetes

Roger A. Clemens

Regulatory Science, School of Pharmacy, University of Southern California, Los Angeles, CA, USA

Abstract

Food-derived peptides, specifically those derived from milk, may adversely affect health by increasing the risk of insulin-dependent diabetes. This position is based on the relationship of type 1 diabetes (T1D) and the consumption of variants A1 and B β-casein from cow’s milk. It appears that β-casomorphin-7 (BCM-7) from β-casein may function as an immunosuppressant and impair tolerance to dietary antigens in the gut immune system, which, in turn, may contribute to the onset of T1D. There are thirteen genetic variants of β-casein in dairy cattle. Among those variants are A1, A2, and B, which are also found in human milk. The amino acid sequences of β-casomorphins among these bovine variants and those found in human milk are similar, often differing only by a single amino acid. In vitro studies indicate BCM-7 can be produced from A1 and B during typical digestive processes; however, BCM-7 is not a product of A2 digestion. Evidence from several epidemiological studies and animal models does not support the association of milk proteins, even proteins in breast milk, and the development of T1D. Ecological data, primarily based on A1/A2 variations among livestock breeds, do not demonstrate causation, even among countries where there is considerable dairy consumption.

Introduction

Bovine milk is a rich source of energy and many essential nutrients, and is often consumed from infancy throughout life. The 2005 United States Dietary Guidelines for American recommends the daily consumption of 2–3 cups of low-fat or fat-free milk or complementary amounts of dairy products, such as yogurt and cheese. Evidence-based health benefits of dairy products have been supported by numerous health organizations and regulatory agencies.
The diabetogenicity of bovine milk protein was suggested based on the observational relationship of its consumption and the apparent incidence of type 1 diabetes (T1D). Subsequent studies suggested the casomorphins derived from specific variants of β-casein may have unique structural properties that, in part, explain their potential bioactivity. One peptide, β-casomorphin-7 (BCM-7) is a peptide derived from the A1 variant of β-casein which is primarily found in high frequencies among Holstein or Holstein-Friesian, Hereford, Ayrshire and Brahman cows. Originating in Europe, approximately 95% of today’s dairy cows in the US are Holstein or grade Holstein.

There are thirteen genetic variants of β-casein in dairy cattle. Among those variants are A1, A2, and B, which are also found in human milk. The amino acid sequences of β-casomorphins among these bovine variants and those found in human milk are similar, often differing only by a single amino acid. Studies indicate BCM-7 can be produced from A1 and B during in vitro digestive processes; however, BCM-7 is not a product of A2 digestion. The evidence of peptide absorption from a normal, healthy human gastrointestinal tract is scant. However, antibodies to this peptide have been detected in individuals with celiac disease and those diagnosed with autism and schizophrenia in which there are several immune and gastrointestinal disorders.

Evidence from epidemiological studies and animal models is inconsistent with respect to the association of milk proteins, even proteins in breast milk, and the development of T1D. Ecological data, primarily based on A1/A2 variations among livestock breeds, do not demonstrate causation, even among countries where there is considerable dairy consumption. Regardless, the implication of variant A1 consumption in the development of T1D remains controversial. This apparent relationship has prompted some changes in livestock composition and subsequent exposure to the A1 and A2 variants. The public health impact on these changes remains to be evaluated.

**Overview Dietary Peptides**

Dietary proteins and the potential health aspects of their respective peptides are of considerable interest among food scientists, health professionals, and pharmaceutical companies. These peptides may be available through several avenues, including hydrolysis during normal digestive processes, proteolytic activity of typical microflora, and fermentation actions facilitated by enzymes from plants and microorganisms.

Depending on their site of action, it may not be necessary for bioactive peptides to cross the mucosal barrier. Some peptides cross the intestinal epithelium via transcellular and paracellular pathways, peptide transport systems as well as through translocation across Peyer's patches. Regardless, many of
these peptides mediate physiological responses at various molecular targets, such as the cardiovascular system, nervous system, digestive system, and the immune system [1] (see fig. 1).

Inhibitory peptides of angiotensin-converting enzyme, a key enzyme in blood pressure regulation, have been identified following hydrolysis of milk, soy and fish proteins. One of the casein-derived peptides, Val-Pro-Pro, may modulate monocyte adhesion and downregulate epithelial inflammation by attenuating the c-Jun NH2-terminal kinase pathway. This peptide may reduce the risk of cardiovascular disease. Pepsin digestion of lactoferrin, a bovine milk protein, can produce lactoferricin, an antimicrobial peptide that, as in

Fig. 1. Food-derived bioactive peptides. From Korhonen and Pihlanto [1], with permission.
vitro studies indicate, may also have immunostimulatory and antiviral properties. The biology of human milk and colostrum demonstrates they are rich in proteins and peptides, including growth factors, hormones, antiviral and antibacterial factors, and even some potential allergens.

Opioid peptides, those which include amino acid sequences that function as opioid receptor ligands that behave as agonists, may be formed from most dietary protein sources, such as milk, grains, legumes, vegetables, meat and poultry. For example, enzymatic digests of milk proteins, such as \( \alpha \)-casein, \( \beta \)-casein, \( \kappa \)-casein, \( \alpha \)-lactalbumin, \( \beta \)-lactoglobulin, and lactotransferrin yield an array of exorphins, casoxins, casomorphins, lactorphins, and lactoferroxins [2]. Some of these peptides have morphine-like activity by binding opioid \( \mu \)-receptors. These receptors are found in many tissues and compartments, such as the central and peripheral nervous system, the endocrine system, and the immune system. The physiological significance of these peptides derived from \( \beta \)-casein is the center of considerable controversy. Some studies suggest this peptide may reduce normal gastrointestinal peristalsis and may ‘trigger’ susceptible individuals to diabetes.

All proteins are subject to variations in structure and amino acid sequence. Bovine milk contains at least four caseins: \( \alpha_s \)-casein, \( \alpha_{\kappa_2} \)-casein, \( \beta \)-casein and \( \kappa \)-casein. Similarly, whey protein is composed of an array of acid-soluble proteins, including \( \alpha \)-lactalbumin, \( \beta \)-lactoglobulin, immunoglobulins, glycomacropeptides, bovine serum albumin and many minor proteins. These kinds of bioactive proteins and peptides are also found in breast milk.

There are thirteen variants of bovine \( \beta \)-casein. Alleles for A1, A2 and B have received the greatest attention for their health implications. The frequency distribution of these alleles is quite broad among cattle breeds. The most common variants among Western cattle are A1, A2, and B. Jersey (~30%), Normande (~45%) and Hereford (~25%) breeds have a relatively high frequency of the B variant. A2 is the dominant allele in Guernsey (>96%) cattle. Cattle breeds such as Angus (~95%), Ayrshire (~60%), Hereford (~75%), Holstein (~60%), and Shorthorn (~49%) primarily present the A1 allele. Nearly all milk-producing breeds contain a blend of the A1, A2 and B alleles [3].

There are several genetic polymorphisms associated with the opioid peptides derived from \( \beta \)-casein. For example, A1 and A2 variants differ by His to Pro amino acid substitution at position 67, respectively. A1 and B variants differ at position 122, where Ser replaces Arg, respectively. In general, the \( \beta \)-casomorphins from these \( \beta \)-caseins are stable to enzymatic degradation. In vitro digestion with pepsin, pancreatic elastase and leucine aminopeptidase of A1 and B yielded BCM-7. A2 is resistant to hydrolysis by these proteases. Thus, depending on the procedures and proteolytic enzymes used to prepare dairy products, such as milks, yogurts, and cheeses, the BCM content may vary and possibly become negligible.
Overview Diabetes

In the US, the crude incidence of diagnosed diabetes increased 136% from 3.3 to 7.8 per 1,000 population between 1980 and 2007 based on CDC statistics. According to WHO, the global burden of diabetes is expected to reach 230 million by 2030, twice the estimated prevalence in 2000. The estimated health care expenditures, depending on the mode of treatment of this chronic disease, exceeded USD 132 billion in the USA in 2002, representing approximately 10% of the health care financial burden. This percentage increases more than twofold among many developing countries. The highest prevalence of diabetes is found in India, China, USA, Indonesia, Japan, Pakistan, Russia, Brazil, Italy and Bangladesh.

T1D, formerly known as insulin-dependent diabetes mellitus, is the second most common childhood malady after allergy. This disease reflects interactions of a constellation of complex factors that ultimately contribute to the destruction of insulin-producing β-cells and the presentation of insulitis and β-cell autoantibodies. Genetic predisposition, environmental factors, and autoimmune mechanisms are involved in the pathogenesis of this immune-mediated disease that eventually leads to insulin deficiency.

Possible dysfunction of the gastrointestinal immune system and stimulation of pancreatic antigens have been suggested to predispose individuals to T1D. Even disruptions in intestinal microflora and a compromised mucosal barrier have been implicated as contributors of decreased tolerances to dietary antigens and consequential autoimmunity that trigger T1D pathogenesis. For example, wheat-derived peptides may initiate intestinal inflammation among T1D individuals by penetrating atypical cell junctions, activating T cells, and interacting with antigen-presenting cells. The ensuing cascade production of inflammatory cytokines, such as γ-interferon and tumor necrosis factor further compromises the gut immune function [4, 5]. While there is evidence that these cereal peptides can initiate or modulate intestinal inflammation among susceptible individuals, some would suggest bovine insulin can trigger an immunologic response in an immature gastrointestinal tract, contribute to an aberrant inflammatory response, and lead to an increased risk of T1D.

Human leukocyte antigen (HLA) haplotypes have been implicated in several autoimmune diseases, including susceptibility to T1D. The frequencies of various DRB1 and DQB1 alleles may confer a predisposition of developing T1D. A 9-year longitudinal study among 254 HLA-DQB1-positive children who received cow's milk during infancy or early childhood demonstrated that this protein source was not associated with a clinical progression of T1D. However, when corrected for several confounding factors, such as genetic susceptibility and age at cow's milk introduction, the relative risk statistics indicated high consumption (>3 glasses/day) of cow's milk can be diabetogenic among those genetically predisposed to T1D [6].
Dietary Peptides and Diabetes

About 25 years ago, the diabetogenicity of cow’s milk hypothesis was advanced based on studies with the diabetes-prone BB (bio-breeding) rat model. This study indicated the 35 and 52% incidence of diabetes among animals fed milk or wheat protein rations, respectively, was reduced to 15% when elemental amino acids replaced the protein sources [7]. Other studies with nonobese diabetic (NOD) mice suggested early introduction of diabetogenic food components even as late as puberty may trigger diabetes onset. Interestingly, the introduction of protein hydrolysates or chloroform-methanol extracted rations virtually precluded diabetes development [8]. A more recent pilot study among HLA-DQB1 allele-positive infants indicated that the early introduction of a casein hydrolysate may modulate β-cell autoimmunity.

Two noteworthy studies among NOD and BB mice yielded inconsistent results. The international study indicated a milk-free, plant-based (primarily wheat) ration was significantly more diabetogenic than rations containing 10% milk hydrolysates. These data also indicated the animals developed diabetes, regardless of the β-casein variant [9]. On the other hand, an earlier study among A1 and A2 variant-fed NOD mice presented a higher incidence of diabetes in the A1 group. Again, diabetes was evident in each study group [10].

Observational studies among Polynesian children in Somoa (formerly associated with New Zealand) in the South Pacific and those in Auckland, Australia, indicated a low incidence of T1D among children who were breastfed or consumed little cow’s milk. Similar assessments among the Maasai in Kenya and northern Tanzania whose diets consist primarily of cow’s milk and maize meal indicated a low incidence of T1D. Interesting follow-up studies with NOD mice indicated those receiving the A2 variant of casein did not develop diabetes.

Several studies among T1D patients and with those diagnosed with celiac disease detected antibodies to bovine β-casein, particularly A1 casein. Celiac disease, previously known as celiac sprue, is an autoimmune disease prompted by undigested gluten, primarily gliadin peptides, from cereal grains, such as wheat, rye and barley. Many of these gluten-intolerant individuals are HLA DQ2-positive and often present symptoms associated with chronic gastrointestinal inflammation during early childhood.

The consumption of cow’s milk with A1 or A2 variants has been compared with the incidence of T1D. In the absence of cheese consumption, data from 16 countries indicate a significant correlation between total milk consumption and the incidence of T1D among males under the age of 15 years [11] (fig. 2). It is important to note that the correlation, \( r^2 = 0.72 \), occurs in the absence of cheese. This correlation diminishes to \( r^2 = 0.23 \) when the consumption of the B variant is included. While these data suggest a strong relationship, it is important to note that there are limited data indicating BCM-7, per se, is
actually produced during in vivo digestion in humans. The in vivo release of this peptide from A1 β-casein is implied by the detection of opioid peptides in blood and urine of autistic and schizophrenic subjects. However, the clinical presentations of these conditions include gastrointestinal dysfunction and altered dietary protein and peptide digestion and absorption.

**Conclusion**

T1D is a chronic autoimmune disorder among genetically predisposed individuals that contributes to the progressive dysfunction of the pancreas, and the inability of the β-cells to secrete insulin. Most of those diagnosed with T1D are children typically before achieving puberty. Among the many hypotheses advanced to describe dietary factors that lead to T1D development is the early exposure to cow’s milk. Animal models, observational studies, and clinical trials to assess the diabetogenic role of β-casein peptides, specifically from A1 and A2 variants, have yielded inconsistent results. Exposure to these variants depends on the livestock breed, the amount of bovine milk consumed and the duration of breastfeeding. Individual genetics and environmental variables may be important T1D predisposing factors. There appear to be dietary components, other than milk, that may trigger or contribute to the onset of T1D. The composite of current evidence from these kinds of studies and factors is insufficient to develop a national health policy, invoke changes in livestock characteristics, and alter early dietary patterns with respect to the consumption of bovine milk [12].
References


Discussion

Dr. Rock: I would like to ask where we can go forward with this information. I am wondering if among the bodies who have prepared recommendations and summary statements, most of them have concluded as you did, that the evidence is not very supportive?

Dr. Clemens: The US has not taken a position on that. Some countries and some regions have actually changed their livestock. I know there is a big push, and I know there is a number of organizations that actually screen their livestock and slowly remove the A1 variant from the breeds, and certainly the dominant part here is A2. So maybe we don’t have long-term data to say that in fact was going to give us the answer. I think there may well be other factors. We have not taken a position on this even though the dairy people are trying to respond to it. I think we just have to keep it in proper perspective. We examined this when I was with a major company of the world over 30 years ago, and we need to examine receptor sites, we need to examine how those receptor sites change over the span of life. We know that different receptors respond differently upon birth vs. later on in life. We certainly know that’s the case with vitamin D receptor, for example, and we certainly have seen that with various hormones. We know that some hormone receptor sites are much more sensitive at birth. We also have configurations of components in the diet that maybe don’t have the same binding affinities at birth as later on in life. So, there are a lot of variables here. I think it’s a bit premature to say the A2 or A1 variant in cow’s milk is the causative
agent. I think it's an interesting concept to examine. I think that at the end of the day we are consuming food, and in a lot of places of the world people are grateful to have some milk to consume.

Dr. Anderson: Dr. Frasier Scott produced several publications on the BB rat, in which he argued that casein was a cause of type 1 diabetes. But I believe that Dr. Scott has changed his opinion as this effect of casein can happen with a number of proteins, when the intestinal barrier is weakened and proteins or peptides affect the β-cell and peptides amino acids.

Dr. Clemens: You remember well, and that's why we are seeing more and more research on the gut barrier function and the gut immune system, because the gut is a great immune organ. I think that's going to be a major focus of future research in this area.

Dr. Thorsdottir: I just want to comment on Dr. Rock's question about cow's milk intake in infancy and the incidence of type 1 diabetes. There are studies from Finland where they are doing long-term interventions and giving the infants who have a genetic risks for type 1 diabetes different kinds of formulas. They actually reported a higher concentration of antibodies in those children who received intact milk proteins. However, if the children had received something else to eat, other proteins, the risk may have been the same, they would have shown other antibodies. So, this is really a difficult task, but if you are interested in milk in general and type 1 diabetes, I would recommend looking into this Finnish intervention.

Dr. Hernell: I was wondering about the association between cow's milk protein and type 1 diabetes in children because if you look at Finland and Sweden, we have a very high incidence that has been increasing for many years, and I don't think that you can see a correlation between the milk intake and the increase in diabetes. Now, at least in Sweden, it seems that the increase has leveled off. Again, I don't think there is a clear association. Also, I find it difficult to believe that a change in the composition of the breed resulting in different casein patterns should be the culprit. It must be difficult to differentiate an effect of milk intake from a general effect on increased growth, which is also associated with increased diabetes risk.

Dr. Clemens: I appreciate that remark. Indeed, this is what I also questioned. Can we change that, and is it going to make a significant difference? I think Dr. Thorsdottir is right in that if it's not milk, it will be something else in our diet that may precipitate these kinds of conditions. I think we have to continue looking at foods without drilling down too far and actually exacerbating and making a public health problem out of it.

Dr. Thorsdottir: I think the problem with the discussion about β-casein A1, β-casomorphins, type 1 diabetes and even the diseases of the central nervous system was that it was too early on the news and too early on the market, even though it was mainly in New Zealand and in Australia. People were too eager to make a lot of money before they had any evidence, and from my point of view this also disturbed the research.

Dr. Netrebenko: As you know, infants born with severe intrauterine growth retardation have a lack of β-cells and nephrons. Do you know whether the lack of β-cells in these infants could result in diabetes?

Dr. Clemens: That's a very interesting question. I am not a diabetologist, I am an endocrinologist, so I can't answer that question. I would be glad to pursue it for you and get back to you on that.

Dr. Hernell: I think with type 1 diabetes there is an association with high birthweight and rapid growth early in life, but I do not remember seeing data on an association with low birthweight.