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# SUMMARY OF PUBLISHED RESEARCH CONCERNING THE CONSUMPTION OF BETA CASEIN A1 AND LINKAGES TO BIOLOGICAL EFFECTS

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## 1. General Introduction

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A2 Corporation Limited holds licensing rights to a portfolio of intellectual property concerning the production and marketing of A2 Milk™ and A2 Milk™ Products.

This document is a review of the published research that serves to support claimed benefits of excluding beta casein variant A1 from milk. The information presented and discussed genuinely represents the views of the company and have been arrived at in good faith and on the basis of the best information available at the date of this document. A2 Corporation has not intentionally omitted the discussion of any studies published to date that it believes counters any belief expressed.

Appended to this summary are a number of articles and studies from scientific publications, cited to provide a comprehensive view of the scientific foundation of A2 Corporation's beliefs.

Based on the science outlined in this report, A2 Corporation presents A2 Milk™ as a value-added product about which a number of claims can safely be made with regard to its potential benefits to consumers. However, due to the likelihood of misinterpretation of claims, it is essential that great care be taken to stick to facts and to avoid generalising claims without qualification.

The contents of this package are collated so as to confer a depth of understanding through the thorough presentation of the underlying science in order that queries and challenges to these claims may be answered with fact from published scientific reports and studies.

Among the claims that may be safely made about A2 Milk™ are the following:

A2 Milk™ is milk from which A1 beta casein, a protein variant found in most milk, has been excluded\*.

A2 Milk™ is not genetically modified, and contains the nutrition and goodness of standard milk.

Epidemiological studies examining the relationship between the consumption of A1 beta casein and the incidence of childhood diabetes in a number of developed countries have revealed a strong statistical correlation.

Similar studies examining the relationship between the consumption of A1 beta casein and heart disease mortality rates across a number of developed countries reveal an even stronger statistical correlation.

Epidemiological studies have linked the consumption of A1 beta casein with the aggravation of neurological disorders such as autism and schizophrenia.

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\* In practice, due to plants that dual process (both standard and A2 Milk) or animal handling error, some carry over A1 protein may be detected in A2 Milk at insignificant levels.

Literature agrees that the digestion of A1 beta casein can result in the production of the bioactive beta casomorphin-7 (BCM-7), this is not the case with A2. This bioactive has been linked to physiological traits that may elicit effects on components of the vascular and immune systems.

BCM-7 is an established and characterised bioactive peptide that exhibits strong opioid activity.

BCM-7; through animal studies, knowledge of its yield and transport and extrapolation of human studies concerning casein feeding, presents itself as a likely agent that aggravates a number of neurological disorders; including symptoms of autistic spectral disorders.

BCM-7 has been shown to oxidise LDL, a determining step in the formation of a recognised risk factor involved in the development of arterial disease.

Differential A1 vs. A2 animal feeding trials have shown A1 beta casein to elicit biological effects in animals which lead to heart disease.

A2 beta casein was the original beta casein gene. Subsequent genetic mutation yielded A1 beta casein. Hence, all milk produced by cattle thousands of years ago, before the large-scale domestication of cows, was A2 Milk.

**A2 Milk™ allows the consumer to choose to avoid the consumption of beta casein A1, and hence the perceived risks associated therewith to date, without giving up the rich source of nutrition that is milk.**

## **2. Biological Effects of beta Casein A1 derived Protein Fragment beta Casomorphin-7.**

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The effect of bioactive protein fragments encrypted in major milk proteins cannot be realised until they are released by enzymatic digestion, either naturally, in the gastrointestinal tract, or by commercial processing. A review published in 2000, titled “*Effects of milk-derived bioactives: an overview*”, presents a summary of peptides encoded in and yielded by milk proteins<sup>1</sup>.

It is thought that steps utilised in milk processing, such as pasteurisation and homogenisation, may affect the casein micelle size and structure thus affecting the digestion and increasing the subsequent yield of bioactives. Additionally, the renneting process involved in the manufacture of hard cheese is thought to crosslink the proteins, effectively inactivating the bioactivity of the resultant digested fragments.

A review published in 2000<sup>2</sup> describes in detail a range of peptides with opioid function that derive from all the casein subgroups;  $\alpha$ -casein,  $\beta$ -casein,  $\kappa$ -casein, as well as from whey fraction proteins  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin and serum albumin. Thus noted, eliminating beta casein from milk would not remove the total theoretical risk of opioid peptide yield.

However, beta casein A1 has been shown to yield the protein fragment, or peptide, termed ‘beta casomorphin 7’ (BCM-7). First reported in 1979<sup>3</sup> as a casein derived opioid, BCM-7 can subsequently be metabolised to beta casomorphin 5 (BCM-5). These peptides have the 3<sup>rd</sup> highest and highest affinity, or binding, to opiate receptors of those reviewed<sup>1</sup>.

BCM-7 has been shown to be significantly more resilient to metabolic processing<sup>4</sup> than other studied food derived exomorphins<sup>1</sup>. Thus, by removing beta casein A1 from milk and thereby eliminating the yield of BCM-7 the risk presented by opioid peptide yield from milk may be significantly reduced.

Both public<sup>5</sup> and private reports to A2 Corporation from consumers known to be prone to exomorphin triggered symptoms of autistic spectral disorders report that A2 Milk™ (A1 excluded) eases their symptoms significantly. Additionally, further reports that individuals exhibiting other undesirable effects following consumption of standard but not A2 Milk can be hypothesised to be casomorphin mediated.

These reports, coupled with published research, lead A2 Corporation to believe that the exclusion of beta casein A1 from milk products may render exomorphin yields physiologically insignificant.

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<sup>1</sup> externally obtained agent with opioid function

## 2.1 Background on Metabolism of beta casomorphin-7 (and truncated forms).

The difference between  $\beta$ -caseins A1 and A2 is in a single amino acid in a strand of 209. Beta casein A1 has Histidine while A2 has Proline in position 67 of the strand. This difference is also displayed between minor variants, which on the basis of this amino acid substitution and known digestion of beta casein variants A1 and A2, may be classified as 'A1 like' or 'A2 like'. These variants include: A3, D and E which, like A2, contain a Proline at this position; and B, C and F which, like A1, contain a Histidine at amino acid residue 67.

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)  $\rightarrow$  CAT (A1, Histidine). Theoretically this difference in amino acid sequence suggests a conformational difference in the secondary structure (folding of the amino acid chain) of the expressed protein, which subsequently may have ramifications on the physical properties of the respective casein micelles.

It is known however that this difference does affect the proteolytic processing of the beta casein in the mammalian gut. *In vitro* studies<sup>6</sup> have shown that the bioactive peptide BCM-7 is yielded by the successive gastrointestinal proteolytic digestion of beta casein A1, but not A2. Derived from residues 60-66 of A1 (and related variants), BCM-7 is produced by A1's successive proteolytic digestion by pepsin, pancreatic elastase and leucine amino peptidase. Previous studies support the theory that this occurs *in vivo*.<sup>7,8</sup>

BCM-7, or further truncated forms such as BCM-4 and BCM-5, have been shown to have a number of effects both *in vitro* and *in vivo*, a selection of which are discussed at the end of this section. It is of note that the BCMs are established opioids, effectors of the  $\mu$ -opioid receptor which is expressed in a broad range of tissues, and have other noted effects on components of the vascular and immune systems.

It must be noted that a significant number of studies have been performed on casomorphins 4, 5, & 6. These are truncated forms of BCM-7, and have very similar properties with regard to opioid like activity. This is supportive of the notion that the 4 NH<sub>3</sub>-terminal residues of BCM 7 (and thus 4-6) are important for  $\mu$ -opiate receptor binding and modulation. A comprehensive, but slightly dated, text<sup>9</sup> collates a number of these studies.

It is thought that biologically available BCM-7 is truncated by exo-peptidases (in the gut, circulation and/or tissue) thus generating BCM's 4-6. Though one cannot state that these casomorphins are absent following the *in vivo* digestion of A2, it would seem plausible that the formation of BCM-7 following A1 digestion may greatly increase the yield and thus bio-availability of these related peptides relative to digestion of A2.

In general beta casomorphins are very stable with regard to enzymatic degradation, prone only to rapid degradation by dipeptidyl-peptidase IV (DPP IV). DPP IV is a cell-surface protease belonging to the prolyloligopeptidase family. It selectively removes the N-terminal dipeptide from peptides with proline or alanine in the second position. DPP IV is expressed on a specific set of T lymphocytes, where it is up-regulated after activation. It is also expressed in a variety of tissues, primarily on endothelial and epithelial cells. A soluble form is present in plasma and other body fluids. It plays a role in glucose homeostasis through proteolytic inactivation of the incretins. DPP IV inhibitors improve glucose tolerance and pancreatic islet cell function in animal models of type 2 diabetes and in

diabetic patients. The role of DPP IV within the immune system is a combination of its exopeptidase activity and its interactions with different molecules.<sup>10</sup>

Two points of note to keep in mind when considering BCM-7 are studies that show that BCM-7 has a long half life relative to other casomorphins, and the knowledge that BCM-7 and the truncated BCM-5 have the strongest relative affinities to opioid receptors<sup>11</sup>. A study on rats, published in the Journal of Dairy Science concluded that beta casomorphin is not likely to be a focus of addiction.<sup>12</sup>

## **2.2 Human beta-casomorphins.**

As by definition previously stated, human breast milk beta casein by virtue of amino acid 67 can be classified as A2, thus it is hypothesised that thermodynamics does not favour the yielding of BCM-7.

Other casomorphins of varying chain lengths can nonetheless be released and it is reported that high concentrations of beta-casomorphin-like peptides are found in the cerebrospinal fluid and plasma of women with postpartum psychosis. Micro-purification of human beta-casomorphin-8 from the milk of a woman with postpartum psychosis is further suggestive of a link<sup>13</sup>.

Human beta-casomorphin-8 (BC8), BC-immunoreactivity (BCIR) was detected in rostro-caudally increasing levels in nineteen microscopically distinct and functionally relevant areas of mesencephalon, pons cerebri, and medulla oblongata of eight infants<sup>14</sup>. Data in the literature, together with those of this study indicate that beta-casomorphins could be transported by specific mechanisms from the blood into the brain stem and that they could play a role in the central regulation of various physiological phenomena.

Regardless of comparative physiological function between human and bovine derived casomorphins, it is important to note that human beta-casomorphin-5 (Tyr-Pro-Phe-Val-Glu) is about ten times less potent than bovine beta-casomorphin-5 (Tyr-Pro-Phe-Pro-Gly)<sup>15</sup>. Furthermore it was noted in this study monitoring the behaviour of rats, that approximately ten times more naloxone was required to antagonize the beta-casomorphin-5 effect than that of morphine.

## **2.4 Physiological characteristics of casomorphins**

It should be stated that currently A2 Corporation acknowledges two primary physiological effects of BCM-7: opiate-like activity, which has been widely reported; and the oxidation of LDL or peroxidation of a lipid component of LDL<sup>16</sup>, regarded as a determining step in the development of heart disease. The former activity may be more readily apparent with respect to physiological response and may serve to facilitate the transport of macromolecules, however it is believed that the latter observed activity of BCM-7 has a much stronger bearing on the development of arterial lesions and plaques.

Absorption may not be necessary to elicit all biological effects. This is suggested by a study reporting that BCM-4 modifies the peristaltic reflex by reducing the efficacy of the reflex response and modulating the timing of the reflex pathway via opiate receptors. This shows that casomorphins could have a local effect on the gut wall with no need for systemic absorption.<sup>17</sup>

The theoretical scope for biological and physiological responses to exomorphins is increased dramatically if they are absorbed into circulation and transported to tissues and organs. This occurrence of such phenomena due to the ingestion and subsequent digestion of beta caseins is supported by a number of reports.

Precursors to BCM-7 have been detected in the plasma of new born cows following milk intake<sup>18</sup> and in new born, but not, adult dogs<sup>19</sup>. Additional to this, larger peptides derived from caseins have been shown to be absorbed and detected in human plasma<sup>20</sup>. This demonstrates the ability of the BCM-7 to be transmitted into circulation following digestion, notably in newborn or young mammals. Studies on the (non BCM specific) trans-epithelial transport of oligopeptides suggest absorption to be at least partially mediated by brush-border peptidases<sup>21</sup>.

Further to this, studies have implicated BCM-7 to be transported across the blood-brain barrier (BBB) by limiting transport systems<sup>22</sup> in a carrier-facilitated<sup>23</sup> manner. An early study had suggested that fragments corresponding to BCMs may cross the breast parenchyma-blood barrier into plasma and subsequently penetrate the blood-brain barrier to reach the central nervous system<sup>24</sup>. Indeed, in a s casomorphins have been implicated as being related to apnea in sudden infant death syndrome.<sup>25</sup>

Expected immediate responses to casomorphin-7 release have been reported in papers concerning the slowing down of down the gastrointestinal transit time (GIT) in rats<sup>26</sup> and dogs<sup>27</sup>. Furthermore, a report concerning a diarrhoea patient provided with calcium caseinate as sole nutrient reported a complete remission of the condition within ten days, speculating that an inhibition of intestinal motility mediated by beta-casomorphins released after hydrolysis of casein in the intestinal tract may be the mechanism involved in this effect<sup>28</sup>. It is worth noting that a linkage has been drawn between consumption of milk and anal fissure in infants when contrasted with those that are breast fed<sup>29</sup>

Opiate attributed effects of BCM-7 and truncated forms include studies that show:

- A modulation of rat feeding behaviour as shown by the attenuation of enterostatin action, which suppresses fat intake<sup>30</sup>. BCM-7 has also been shown to stimulate food intake generally<sup>31</sup>.
- The neonatal sleep patterns of rats have been shown to be affected by BCM-7.<sup>32</sup>
- Intracerebroventricular (but not intraperitoneal) injection of beta-casomorphin depresses ventilation in preterm newborn rabbit and adult rats.<sup>33</sup>
- BCM-7 produces a delayed anxiolytic effects when administered systemically to rat pups.<sup>34</sup>
- Casomorphins induce a strong mucin secretion in rat jejunum (response at 563% of controls) that is inhibited by naloxone. Concluded to be triggered by a neural pathway and mediated by opioid receptor activation.<sup>35</sup>

A number of other effects have also been reported to be imparted by BCM-7 specifically, or by related truncated BCMs, some which are also shown to be opiate receptor linked:

- Pseudoallergic skin reaction studies in healthy children indicate that BCM-7 is a non-cytotoxic histamine releaser in humans<sup>36</sup>. The intradermal injection of an opiate receptor antagonist, naloxone, inhibited *in vitro* histamine release and skin reactions only in a 100-fold excess over beta-casomorphine-7. These findings suggest that beta-casomorphin-7 can be regarded as a non-cytotoxic, direct histamine releaser in humans.<sup>37</sup> BCM-5 been reported to cause concentration-dependent *in vitro* histamine release from peripheral leukocytes of healthy adult volunteers.
- (in vitro) BCM-7 affects the human mucosal immune response as represented by depression of lamina propria lymphocyte (LPL) proliferation. The anti-proliferative effect of BCM was reversed by the opiate receptor antagonist, naloxone.<sup>38</sup>
- In studies on human polymorphonuclear leukocytes (PMNs), Tyrosyl – prolyl – phenylalanyl - proline ( $\beta$ CM-4) treatment inhibited oxygen-free radical production and 5-HETE production.  $\beta$ CM-4 seemed to act on the enzymatic activities of endogenous peroxidases that reduced hydroperoxyeicosatetraenoic acid to 5-HETE.<sup>39</sup>
- Casomorphins shown to inhibit both aggregation of ADP-treated platelets and binding of fibrinogen to ADP-treated platelets<sup>40</sup>,
- Shown to affect immune action by stimulating human peripheral blood lymphocytes proliferation at high concentrations and suppressing proliferation at low concentrations.<sup>41</sup>
- BCM-7 plays a role in the regulation of insulin formation in dependence of the glucose concentration as well as insulin secretion from the islets of Langerhans.<sup>42</sup>
- In non lactating dairy cows, injection of beta-casomorphins 4, 5 & 7 significantly lowered the responses of serum insulin to both abomasal and intravenous glucose infusions ( $P < 0.05$ ).<sup>43</sup>

### **3. The link between Beta-Casein A1 consumption & Ischaemic Heart disease/atherosclerosis**

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This section summarises and presents references to a number of studies, which report connections between the consumption of milk protein variant beta casein A1 (A1) and ischaemic heart disease (IHD).

The principal publications regarding the connection between A1 and IHD can be divided under the following subheadings:

- 1) Epidemiology comparing the per capita intake of A1 as compared with the mortality rates for IHD, both within a country and across a number of countries,
- 2) Animal feeding studies investigating the differential effects of feeding A1 vs. A2 casein diets to NZ white rabbits,
- 3) Other studies that lend evidence to the link between A1 consumption and the development and/or progression of heart disease.

#### **3.1 Epidemiology**

The connection between beta-casein A1 consumption and heart disease was first drawn following epidemiological studies<sup>44</sup>. In 1995 Dr McLachlan noted a strong correlation between IHD and A1 consumption ( $r^2 = 0.86$ ), both between countries and within a country (where the mean A1 content of milk & thus the per capita consumption varies between regions).

In January 2003, Laugesen and Elliott published an epidemiological study concluding that A1  $\beta$ -casein per capita supply in milk and cream (A1/capita) was significantly and positively correlated with IHD in 20 affluent countries five years later over a 20-year period.<sup>45</sup>

#### **3.2 Animal Feeding Trials**

Supporting the early epidemiological work, was the observation that the total casein, in comparison to other sources of dietary protein, is a potentiator of heart disease, or related conditions such as hypercholesterolemia, in rabbits<sup>46,47,48,49,50</sup>, pigs<sup>51</sup> and monkeys<sup>52,53</sup>. However, no work had at this stage been performed to identify what element(s) of casein, which includes alpha, beta and kappa caseins and variants thereof, is atherogenic.

In 2002, a rabbit feeding study was commissioned by A2 Corporation and performed by Prof. Julie Campbell at the Wesley Research Institute (Brisbane). The study showed a causative link between A1 consumption and the development and progression of arterial lesions. This work was published by the journal *Atherosclerosis* online in May 2003, and in print in September 2003<sup>54</sup>.

This study showed that in the NZ White rabbit model for heart disease, Relative to A1, consumption of A2\*:

- Reduces serum cholesterol levels
- Reduces circulating triglyceride levels
- Reduces LDL concentrations and thus LDL:HDL ratios.
- Does not promote significant lesion formation or stenosis (thickening) of vessels.
- Does not promote further thickening of previously damaged vessel walls.
- Is atheroprotective, as shown by the apparent prevention of damage to vessel walls induced by addition of dietary cholesterol.

Furthermore, it showed that A2 has an apparent protective effect as indicated by:

- Depressed levels of risk factors, namely serum cholesterol and LDL:HDL ratios. (Figs 1& 2)
- Inhibition of fatty streak development in juvenile arteries, and thereby inhibiting the progression of atherosclerosis in adults.
- Inhibition of atheroma development/progression.

### **3.3 Further Studies**

Further to epidemiological studies and animal feeding trials, work on beta casomorphin 7 has been shown to affect processes involved with arterial disease – these are outlined in section 2, ‘Physiological/bioactive effects of Beta casomorphin-7’.

Of particular relevance is the observation that BCM-7 has been shown to catalyse the oxidation of LDL in a non cationic dependent fashion<sup>55</sup>.

In support of conclusions drawn by Professor Campbell are published observations that cows milk infant formula fed infants raise antibodies to oxidised LDL at 10 times the rate of those who were breast fed<sup>56,57</sup>. This also adds further evidence to the belief that consumption of beta casein A1 may lead to the oxidation of LDL and thus contribute to the progression and development of arterial plaques and thus IHD.

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\* “A1” corresponds to milk proteins (whey and casein fractions) with A1 as beta casein component; “A2” corresponds to milk proteins with A2 as beta casein component – i.e. A2 Milk proteins.

#### **4. The link between Beta-Casein A1 consumption & Type 1 Diabetes**

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This section summarises the reported linkages between the consumption of milk protein variant beta casein A1 (A1) and development or incidence of type 1 diabetes; also known as Insulin dependent diabetes mellitus (IDDM), diabetes mellitus 1 (DM1), or childhood onset diabetes.

The linkages can be divided into three categories as follows:

- 1) Epidemiology, which involves calculating the per capita intake of A1 (by way of milk consumption coupled with either milk analysis or herd gene frequency data) before comparing it with the incidence of disease;
- 2) Studies which test A1 → IDDM hypothesis by feeding trials utilising animal models of disease; and
- 3) Other studies which include those that link beta caseins to immune response.

Although a number of animal feeding trials have produced results which support a causal relationship between A1 consumption and the development of diabetes some investigations have been inconclusive. It could be reasoned that this is the case due to two possible causes:

- i) The inadequacy of the model to replicate the disease state in humans. Both the NOD mouse and BB rat models develop diabetes spontaneously through genetic deficiencies. One cannot assume these are the same as those in humans which are yet to be completely discovered or understood.
- ii) The diets which the animals were fed may not have been fully defined with regard to their exogenous opioid content. Such agents have been implicated in disease causation and it is known that similar agents are derived from cereals. Furthermore, in an internal report produced by a group associated with the 2002 study<sup>58</sup>, it was noted that the hydrolysed protein (Pregestimal), which was used in this study as a control and supplement, contained high levels of the exogenous opioid beta casomorphin 7.

It could be observed that there are dual hypotheses with regard to the causative role of beta casein variants on the development of IDDM: one involving the opioid activity exhibited by BCM-7; the other concerning a possible antigenic determining role of beta casein in auto immune reactions.

Regardless, the firm relationship suggested by two independently performed epidemiological studies is not only consolidated by results from animal trials but also by a rapidly developing understanding of A1 derived BCM-7 and its biological characteristics (see section 2). The most well characterised function of this peptide is as an effector of opiate receptors, however it has also been shown to catalyse the oxidation of LDL and elicit effects not attributed to opioid function.

## 4.1 Epidemiology.

An interest in the possibility that milk proteins may play a role in IDDM began in 1984 with the observed increase in its incidence among Western Samoan children coming to live in New Zealand. A review of contrasting environmental factors indicated that a significant difference in their diet was that the children consumed more cows' milk in New Zealand than in Western Samoa.<sup>59</sup>

Elliott *et al.* (1999) reported a regression coefficient of 0.72 in the plotted linear relationship between A1 consumption and incidence of IDDM. This relationship is stronger than that exhibited by other individual milk proteins, or the combined total milk proteins.<sup>60</sup>

Two years later, McLachlan (2001) published a paper pointing out that the calculated consumption of A1 across 16 countries correlated strongly ( $r^2 = 0.75$ ) with the incidence of IDDM in males under 15 years old<sup>61</sup>. In 2003, Laugeson and Elliott, examining the same relationship between A1 and IDDM, ratified this observation across 20 countries.<sup>62</sup> The latter of these two reports attracted an editorial in the NZ Medical Journal which encouraged that the presented hypotheses concerning A1 consumption and both IDDM and IHD be investigated further<sup>63</sup>.

A paper published in 2002 concerning the measured levels of a number of milk protein variants across 5 Nordic countries compared with the incidence of IDDM concluded that beta casein A1, and not other milk proteins examined (bovine serum albumin, immunoglobulin G, lactoferrin), may contribute to observed variation in the diabetogenicity of cow's milk<sup>64</sup>. This paper stemmed from a doctoral thesis, which reported that the consumption of A1 by children (as opposed to adolescents) showed a regression coefficient of 0.9 ( $r^2 = 0.81$ ) in its relationship to type 1 diabetes<sup>65</sup>.

## 4.2 Animal Trials

An early animal study examining the possibility of a relationship between milk protein consumption and diabetes was conducted by Elliott and Martin (1984). This study involved feeding BB rats with milk protein or an amino acid diet. Elliott and Martin concluded that cows' milk may contain a diabetogenic agent<sup>66</sup>. A further study by Elliott conducted in 1992, showed that rather than all milk being causative of Type 1 diabetes, it was possible that there was a link between digested casein, and more specifically a hexapeptide peptide derived from digested beta-casein protein, and Type 1 diabetes<sup>67</sup>. The implication of a bioactive protein fragment generated by the digestion of beta casein A1 was then reported in 1995<sup>68</sup>, when, in an article title referring to beta casein variants as 'Jeckyl & Hyde', it was further noted that this peptide bound macrophages and had known immunological activity.

Supplementing previous research concerning the link between the A1 variant of the beta casein protein and Type 1 diabetes was a further study presented by Elliott in 1997, which reported that mice fed beta-casein A1 developed high levels of Type 1 diabetes, whereas those fed beta casein A2 did not<sup>69,70</sup>. This study further implicates BCM-7 and its associated opioid activity as it reports that the diabetogenicity is attenuated by naloxone, a specific opiate blocker.

BCM-7 was further implicated as the causative agent in a report published in the journal *Diabetologia* in 1999<sup>71</sup>. A comprehensive summary of BCM-7 can be found in Section 2.

A publication encompassing a range of trials involving both the BB rat and NOD mouse, animal models of diabetes, was published in 2002<sup>72</sup>. Its findings with regard to disease causation were inconclusive (discussed in Introduction to section). However, it did consider the possibility that, although it seems unlikely that a casein variant is an exclusive promoter of diabetes, it may enhance the outcome in some cases. It is of interest that wheat proteins were exemplified as promoters of diabetes in the conclusion of this paper, as wheat gluten, like A1, is a known source of a food derived exogenous opioid.<sup>73</sup>

### 4.3 Other Reports

Supporting the notion that some children may be more prone to A1 facilitated development of Type 1 diabetes, a genotype was identified that appears to predispose infants who have a high consumption of cows' milk to the development of IDDM<sup>74</sup>.

A review by Pozzilli, published in 1999, considered beta casein as a major antigenic determinant for type 1 diabetes. Although he was not considering particular variants (i.e. A1 or A2), Pozzilli stated that beta casein is a good candidate milk component to explain the observed association between cow's milk consumption on IDDM.<sup>75</sup> This assertion was based on the hypothesis that the immune system produces antibodies to epitopes of beta casein, or fragments thereof, that are homologous to regions of GLUT2 (glucose transporter) on the beta cells of the pancreas.

Modifications to protein secondary structure imparted by single amino acid differences (such as between A1 and A2 variants) may warrant some consideration. More specifically, a small change in structure may expose an epitope in A1 which initiates the formation of autoantibodies.

A subsequent familial study measured and differentiated antibodies against A1 and A2 casein variants in four groups of people: IDDM patients, their siblings, individual parents, and healthy controls. It was found that antibodies against casein were present in all four groups, with the highest titres being found in diabetics. Increased amounts of anti-casein A1 antibodies were found among patients with IDDM and their siblings. On the other hand, the serum samples from parents and control persons contained antibodies against the A2 variant. This observation serves to underpin the linkage between A1, the exposure revealed by antibody response, and diabetes.<sup>76</sup>

The above studies were later supplemented by a publication addressing beta casein as an antigenic determinant for a range of auto immune disorders. It concluded that beta casein may be relevant in the development of autoimmune diabetes.<sup>77</sup>

## **5. The Link between A1 Consumption and Neurological Disorders.**

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A number of studies have been published regarding the connection between diet and neurological disorders. The sum of these studies indicates that beta casein A1 may aggravate some neurological disorders. The studies can be divided into a number of sub-categories:

- 1) Epidemiological studies
- 2) Human Studies
- 3) Animal Trials
- 4) Studies on BCM-7

Citing a cross-section of these reports, an abandoned patent application filed in March 2002<sup>78</sup> explicates the connection between beta casein A1 and neurological disorders and concludes that supplying milk from A2/A2 cows could be presented as “a method of providing nutrition to a susceptible individual while avoiding the induction or aggravation of a neurological/mental disorder”.

As discussed in previous sections, it is well established that exogenous opioid BCM-7 is uniquely yielded from the digestion of A1. Results from animal trials and human studies implicate food-derived opioids as agents that aggravate a range of neurological conditions, and whose action is reversible by the administration of specific opiate blockers.

### **5.1 Epidemiological Studies**

Epidemiological studies were presented in this patent application that examined the connection between deaths due to mental disease and the consumption of beta casein A1. The analysis reveals a ‘statistically relevant’<sup>1</sup> correlation with beta caseins with a histidine at position 67 (‘A1-like’), while neither total milk proteins nor beta casein A2 and A3 exhibited a statistically relevant correlation.

### **5.2 Human Studies**

Prior to the submission of the patent application, tests for BCMs in urine samples from autistic children and age-matched normal children were performed. These tests showed that, while normal children did not have beta casomorphins in their urine, the autistic children did. Small amounts of beta casomorphins were detected in the urine of the autistic children after consuming milk containing beta casein with proline at position 67 (‘A2-like’), however, the amount of beta casomorphin-6 measured in the urine increased ten-fold when their diet was changed to milk containing beta casein with histidine at position 67 (‘A1-like’). This trial supports the hypothesis presented in section 2, that milk from A2/A2 cows,

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<sup>1</sup> As defined in ref. 78, p. 8, lines 8-17.

which contains only beta caseins with proline at position 67, does not release the bioactive peptide BCM-7 or its analogues.

There are a number of laboratories around the world that offer testing services to measure casein and gluten intolerance (levels of BCMs, and gliadinomorphin in the urine), or the levels of antibodies to gliadin or casein. These tests are used as an indication of the likely benefits of dietary intervention (e.g. the removal of grains and/or milk from the diet).

Professor Cade of the University of Florida, who has contributed to a number of studies on autism, has provided preliminary results from a behavioural study of 70 patients with childhood autism who were put on a gluten free, casein free (GFCF) diet. The results from Cade’s observations are as follows:

Table 1: Mean score during the control period and while on a GFCF diet for Nine characteristics that occur frequently in autistic children.

Legend: 0 = normal, 1 = Good, 2 = Fair, 3 = Poor, and 4 = Very Poor

<b>Characteristic</b>	<b>Control</b>	<b>1 month</b>	<b>3 month</b>	<b>6 month</b>	<b>1 year</b>
<b>Social isolation</b>	3.5	2.9	1.9	1.4	1.4
<b>Eye contact</b>	3.2	2.4	2.7	1.2	1.2
<b>Mutism</b>	2.8	2.1	1.5	0.9	0.8
<b>Hygiene</b>	1.9	1.9	1.2	1.2	1.2
<b>Learning skills</b>	3.3	2.8	2.1	1.6	1.2
<b>Hyper-activity</b>	3.1	2.1	1.7	1.6	1.5
<b>Sterotypical activity</b>	3.3	2.8	2.1	1.5	1.1
<b>Self mutilation</b>	0.63	0.57	0.37	0.47	0.30
<b>Panic attacks</b>	1.5	1.3	0.80	0.83	0.45

These results indicate that a gluten free, casein free diet has significant positive effects on the behaviour of autistic children.

The University of Florida web site states that the preliminary findings of a recent study showed that 95% of 81 autistic children had 100 times the normal levels of milk protein in their blood and urine. The website points out that, although the results have not been published, they have been presented at two international meetings. Professor Cade is quoted, “When these children were put on a milk free (note, milk free only, not GFCF) diet, at least 8 out of 10 no longer had symptoms of autism or schizophrenia.”

There has also been recent concern in the UK regarding links of autism to cow’s milk. Mr. Paul Shattock the Director of the Autism Research unit at the University of Sunderland has also been reviewing recent research confirming that in some children fragments of casein can leak through the gut wall into the blood, and from there into the brain causing significant behavioural problems. A2 Corporation Director, Dr. Jock Allison, has been in contact with Mr. Shattock, and finds that he is in agreement with the hypothesis that drinking A2 milk, thereby eliminating BCM-7 from digestion, is likely to be an effective means of alleviating these problems.

### 5.3 Animal Trials

Researchers at the University of Florida have conducted a study involving the dosing of rats with varying levels of BCM-7. The study showed that this protein fragment was taken up in the specific regions which have been shown to be altered in patients with schizophrenia and autism<sup>79</sup>. Furthermore, Sun and Cade observed a range of erratic behaviours in rats dosed with BCM-7, which did not occur when the rats were pre-treated with naloxone, an opioid antagonist<sup>80</sup>. These studies suggest that BCM-7 may play a role in behavioural disorders such as schizophrenia and autism.

#### **5.4 BCM-7**

In section 2, material was presented that indicates that beta casomorphin-7 is produced during the digestion of A1, or A1-like, beta casein, but not during the digestion of A2, or A2-like, beta casein. Thus A2 milk, in which the beta caseins consist almost exclusively of the 'A2-like' variant, will significantly reduce, if not eliminate the negative effects of milk attributed to the bioactive BCM-7.

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