

Beta-casein Variants

Beta-casomorphin-7 and Infants

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Hypotheses concerning biological responses to A1 beta-casein cows' milk protein pivot about the release of the exogenous opioid peptide, beta-casomorphin-7 (BCM-7) via the incomplete digestion of A1 beta-casein⁽¹⁻³⁾ and its subsequent absorption into circulation. BCM-7 production is influenced by the amino acid present at position 67 of the beta-casein protein chain⁽⁴⁾. A1 beta-casein has a histidine at this position while the other major beta-casein variant, the A2 beta-casein type has a proline at position 67⁽⁴⁾. Due to this amino acid variation, A1 beta-casein releases BCM-7 following normal enzymatic digestion^(1-3,5,6), whereas A2 beta-casein does not or if it does, it happens at a very low rate⁽⁴⁾.

Opioid peptides play a role in various biological processes, including respiration, analgesia, constipation and behaviour⁽⁷⁾. However, as noted

by Maklakova et al. (1993) "Depending on the type of peptide, the mode of its administration into the organism, the species of experimental animal, and the peptide dose, it is possible to register an entire spectrum of effects, ranging from significant motor excitation to a complete inhibition of locomotor activity and catotonia"⁽⁸⁾.

A1 beta-casein is present in cows' milk and its products, including infant formula but today some cows are selected for their genes to produce only the A2 type beta-casein type and not the A1 type and these cows produce milk which is A1 protein free (e.g. a2 Milk™ in Australia). Notably, human beta-casein and A2 beta-casein share a proline at their aligned residues⁽⁹⁾, which highlights that human beta-casein is of the A2 beta-casein type and not A1 (Figure 1).

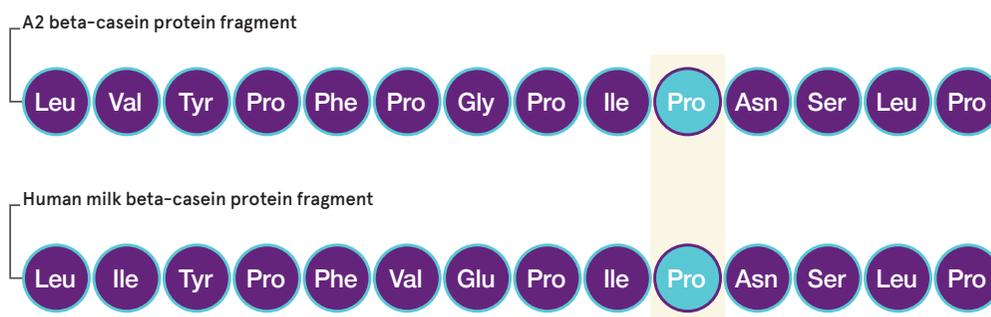


Figure 1: Human beta-casein and A2 beta-casein share a proline at their aligned residues

Human BCM-7 has been identified in human breast milk, which shows that human BCM-7 is present in breast milk before any digestion of the human A2 beta-casein type in an infant's gut⁽¹⁰⁾. Other human beta-casomorphins have also been found in the blood of pregnant and lactating women⁽¹¹⁾, but not in men or non-pregnant women, leading researchers to suggest that human BCM-type mammary products have physiological importance during pregnancy

or after parturition^(10,11). Importantly, human and bovine BCM-7 differ by two amino acids at positions four and five of the peptide (Figure 2). These structural differences affect the opioid activity of BCM-7⁽⁹⁾ with bovine milk beta-casomorphins shown to be at least ten times more potent (i.e. greater binding affinity to mu-opioid receptors) than human beta-casomorphins⁽¹²⁻¹⁴⁾. **This appears to have consequences on the function of each variant.**

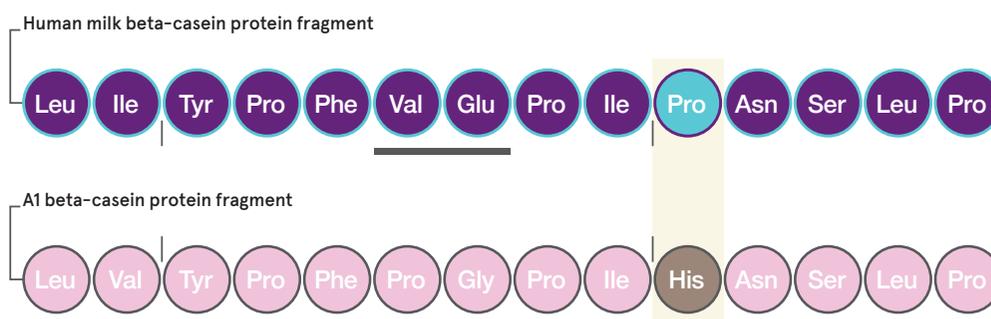


Figure 2: Human derived BCM-7 structure [H-Tyr⁶¹-Pro⁶²-Phe⁶³-Val⁶⁴-Glu⁶⁵-Pro⁶⁶-Ile⁶⁷-OH] versus bovine A1 beta-casein derived BCM-7 structure [H-Tyr⁶⁰-Pro⁶¹-Phe⁶²-Pro⁶³-Gly⁶⁴-Pro⁶⁵-Ile⁶⁶-OH].

Recent studies have found *bovine* BCM-7 in the blood of human babies^(15,16), while earlier research detected beta-casomorphin materials in the cerebrospinal fluid and blood of some lactating women and some female controls⁽¹⁷⁾. The full implications of bovine BCM-7 absorption into human infant circulation requires ongoing research. However, elevated circulating human BCM-7 has been correlated with beneficial developmental outcomes in breastfed infants, while the opposite has been observed in their formula fed counterparts with elevated bovine BCM-7 levels⁽¹⁶⁾. In addition, Jarmolowska et al. (2007) have shown that the concentration of human BCM-7 in colostrum (produced in the first days of lactation) is seven times higher than in human milk at two months of age⁽¹⁰⁾, which suggests that a human infant's requirement for human BCM-7 is significantly lower at two months compared with the first few days of life and that exposure to the more potent bovine BCM-7 at around two months of age may be undesirable.

Binding and rodent studies suggest that human and bovine BCM-7 may have central and/or peripheral effects via their ability to modulate the serotonergic system, in addition to their known effect on the opioid system⁽¹⁸⁻²⁰⁾. Earlier rodent studies suggest an effect of shorter bovine BCM-7 derivatives on dopamine-mediated processes^(21,22). More recent animal research suggests that bovine BCM-7 may affect neurochemically mediated maternal-offspring bonding^(8, 23-25) and learning and environmental adaptation behaviour in infant and young mammals⁽²⁶⁻²⁸⁾.

Currently, the role of *bovine* BCM-7 in the health and development of human infants is the topic of extensive scientific debate. Such debate was stimulated with the study by Kost et al. (2009), which showed that bovine BCM-7 could be measured in the blood of infants fed beta-casein containing infant formula⁽¹⁶⁾. They also reported that higher blood levels of bovine BCM-7 found in some infants was correlated with delays in psychomotor development⁽¹⁶⁾. Furthermore, babies who were unable to metabolise BCM-7 rapidly and thus eliminate it from their systems quickly, were particularly at risk.

Prior to the research by Kost et al. (2009), it had been argued that BCM-7 did not pass into human blood due to rapid proteolysis⁽²⁹⁾, even though BCM-7 and other related immunoreactive materials had been found in the blood of newborn calves⁽³⁰⁾ and dogs⁽³¹⁾. In adult human males, it has also been shown that following caseofamide ingestion (a synthetic beta-casomorphin analogue), caseofamide-like-immunoreactivity was detected in blood within 60 minutes⁽³²⁾. Taken together, these data suggest that mechanisms exist by which beta-casomorphins are absorbed into the blood of both infant and adult humans. There are a range of physiological mechanisms by which this might occur⁽³³⁻³⁸⁾. Further, the transport rate of beta-casomorphins has been shown to accelerate with increasing hydrophobicity⁽³⁷⁾, which suggests a faster rate of transfer for the more hydrophobic bovine relative

to human beta-casomorphins. However, the precise mechanism of beta-casomorphin transfer from the gut to circulation has yet to be determined.

The latest research by a Polish research group⁽¹⁵⁾ confirms the presence of BCM-7 in the blood of babies, but also suggests that BCM-7 may be a risk factor for apnoea (expressed as "apparent life threatening events"), compared to a group of healthy infants. In this study, blood BCM-7 levels in "at risk" apnoea infants were on average three times higher compared with normal infants. This work is consistent with earlier animal studies that have shown BCM-7 induces apnoea and irregular breathing in newborn rabbits and adult rats⁽³⁹⁾, and that the beta-casomorphin analogue morphiceptin has an effect on the brainstem respiratory center in dogs⁽⁴⁰⁾. **Notably, mu-opioid receptors are expressed heavily in the brainstem⁽⁴¹⁾ and BCM-7 is a known mu-opioid receptor ligand⁽⁴²⁻⁴⁵⁾.** This research also identified that "at risk" infants had low blood levels of DPP-4, the enzyme that metabolises BCM-7⁽⁴⁶⁻⁴⁸⁾. In the "at risk" infants, DPP-4 levels were 58% of those found in healthy babies. Furthermore, in the normal infants, BCM-7 was positively associated with DPP-4 levels (which leads to faster BCM-7 elimination). This relationship is absent in the "at risk" babies. The statistics associated with these findings are strong (i.e. $p < 0.001$ in each case). Not surprisingly, this study also showed that BCM-7 levels were higher in babies fed formula which was high in casein, compared to those fed formula that was low in casein (BCM-7 can only be derived from casein and not from other milk proteins). However, it could be regarded as surprising that bovine BCM-7 was also found in the blood of 1-4 month old babies who were breastfed. **The suggestion by the researchers is that bovine BCM-7 from milk ingested by lactating mothers can be transferred directly to the mothers' milk.**

Other research in human infants conducted by a Czechoslovakian research group supports the idea that bovine BCM-7 may be an early contributor to heart disease later in life. Steinerova et al. (2004) have shown that formula fed infants (beta-casein containing formula) have elevated serum levels of antibodies to oxidized LDL relative to breastfed babies⁽⁴⁹⁾. Given that BCM-7 has been shown to oxidise LDL *in vitro*⁽⁵⁰⁾, it is possible that the differences in oxidised LDL between the formula and breastfed babies are mediated via A1 beta-casein derived BCM-7. Although the researchers in this study did not assess serum BCM-7 levels, they did suggest that "As human milk does not contain beta-casein A1 and infant formulas are based on bovine milk, we can express a hypothesis that beta-casein A1 is the substance, which caused increased production of IgoxLDL"⁽⁴⁹⁾.

The same research group has also identified that antibodies to oxidized LDL in formula fed infants have high affinity to A1 beta-casein and BCM-7 relative to A2 beta-casein and maternal milk *in vitro*⁽⁵¹⁾, but the significance of this remains to be elucidated.

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Given the growing body of evidence surrounding the significant health implications of A1 beta-casein derived BCM-7, the call for this issue to be taken

seriously and thoroughly examined impartially and without industry interference is compelling.

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T: +61 (0)3 9249 9703 PO Box 180
F: +61 (0)3 9859 0142 Kew East VIC 3102
www.a2milk.com.au Australia

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