

BETA-CASEIN AND DIGESTIVE, RESPIRATORY, AND IMMUNE FUNCTIONS

Executive summary

Cow's milk and dairy products are a major food source. A major protein component of cow's milk is β -casein, of which there are two primary variants, A1 and A2. Digestion of A1 β -casein yields the peptide β -casomorphin-7, an exorphin. β -casomorphin-7 can target opioid receptors in various systems to influence digestion, respiration, and immunity. Consumption of A2 β -casein instead of A1 β -casein could have beneficial effects on these systems by avoiding the unwanted effects of the latter.

Research highlights

- Digestion of A1 β -casein, but not that of A2 β -casein yields β -casomorphin-7 (BCM-7), an exogenous opioid peptide (exorphin) that can potently activate opioid receptors throughout the body
- Opioid receptors are important regulators of gastrointestinal function, including motility, mucus secretion and hormone/incretin secretion
 - Casein and its derivatives, including BCM-7, slow gastrointestinal motility, which may cause constipation
 - BCM-7 significantly increases intestinal mucus secretion via opioid signalling pathways, which may influence commensal bacteria and drug absorption
 - Unlike A2 β -casein, A1 β -casein delays gastrointestinal function, has pro-inflammatory effects, and enhances the expression of dipeptidyl peptidase 4, a clinically relevant enzyme involved in glucose homeostasis
 - The effects of A1 β -casein on gastrointestinal function and inflammation are at least partly mediated by opioid receptors and the Th2 pathway
 - Reducing A1 β -casein consumption or replacing A1 β -casein with A2 β -casein could alleviate gastrointestinal disturbances
- Many immune cells express the μ -opioid receptor, which has immunosuppressive activity *in vitro* and *in vivo*
 - BCM-7 disturbs immune cell function *in vitro*
 - Consumption of products containing A2 β -casein, which does not yield BCM-7, instead of those containing A1 β -casein, could avoid disruption to immune cell function leading to symptoms of food intolerance

- Opioid receptors play an important role in controlling respiration
 - Activation of opioid receptors in the brainstem, particularly the pons, can suppress respiration
 - BCM-7 and other opiates may be involved in apparent life-threatening events in infants

Introduction

β -casein is a major protein expressed in human and cow's milk and is present in many food products derived from milk. In cow's milk, two primary variants of β -casein, termed A1 and A2, and several much rarer sub-variants have been identified. A1 and A2 β -casein differ in their protein structure by a substitution of the amino acid at position 67. A1 β -casein contains a histidine residue at this position, which allows cleavage of the preceding seven amino acid residues, generating the peptide β -casomorphin-7 (BCM-7). A2 β -casein contains a proline residue at this position, which prevents cleavage of this peptide.¹ The sub-variant B β -casein also has a histidine at position 67, and its cleavage also results in the generation of BCM-7, but this variant is much less frequent than A1 or A2 β -casein in the milk of cows of European origin.

BCM-7 can cross the gastrointestinal wall to enter the systemic circulation and influence systemic and cellular activities via opioid receptors. Moreover, BCM-7 and other derivatives of β -casein are potent exogenous agonists—exorphins—for opioid receptors, with the greatest affinity for μ -opioid receptors.²

Opioid receptors are expressed in many organs, notably the gastrointestinal tract,^{3, 4} immune cells,^{5, 6} and the central nervous system, particularly in the regions controlling respiration.⁷ Activation of these receptors can have unwanted or unexpected effects on gastrointestinal, immune, and respiratory functions. Therefore, it is important to evaluate the potential effects of BCM-7 and related milk-derived peptides on the functions of these systems.

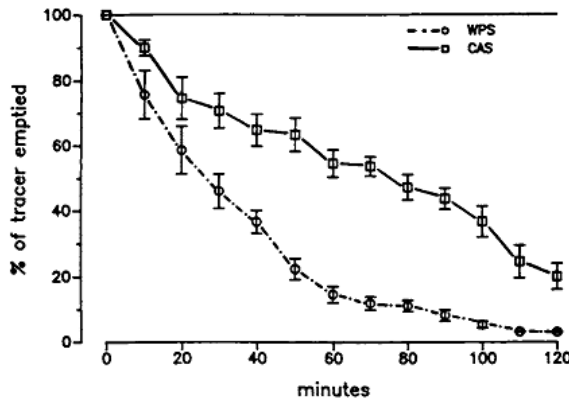
Effects of BCM-7 on intestinal motility

Opioid receptors play a physiologically important role in controlling gastrointestinal function, including regulating gastrointestinal motility, mucus production and hormone/incretin production. Several studies have provided direct evidence that casein and its derivatives, particularly BCM and related peptides, decrease gastrointestinal motility (Figure 1), in part by reducing the frequency and amplitude of intestinal contractions.⁸⁻¹² By contrast, soy protein, which cannot be cleaved to form BCMs, does not exert these effects.⁹ BCM-7 in particular can mimic the effects of opioids, in some cases leading to constipation. The role of opioid receptors in mediating these effects was confirmed, as co-treatment with an opioid receptor antagonist suppressed the effects of casein.⁹

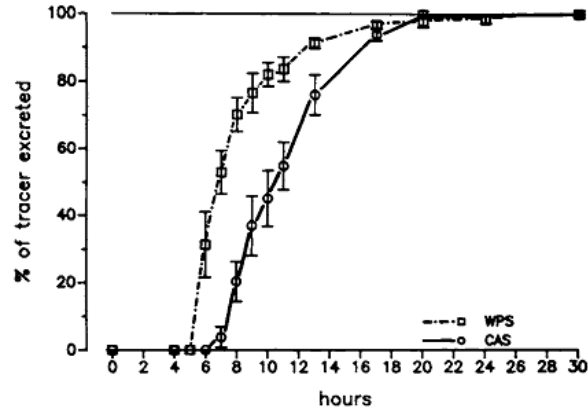
Figure 1. A casein protein suspension delays gastric emptying (A) and protracts gastrointestinal transit time (B) compared with a whey protein suspension in rats. (C, D) Naloxone, a specific opiate receptor antagonist, partially or completely reversed these effects of the casein suspension, revealing the opioid activity of casein-derived peptides. Reprinted from Daniel et al (1990).¹²

CAS = casein protein suspension; WPS = whey protein suspension.

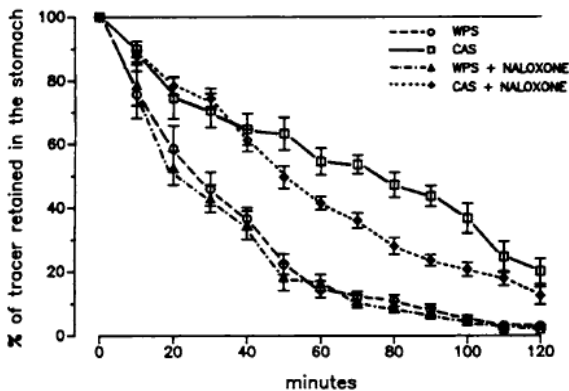
A



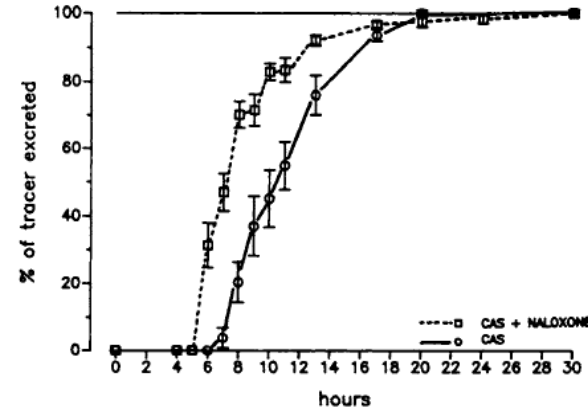
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D



Effects of BCM-7 on the gut's immune system

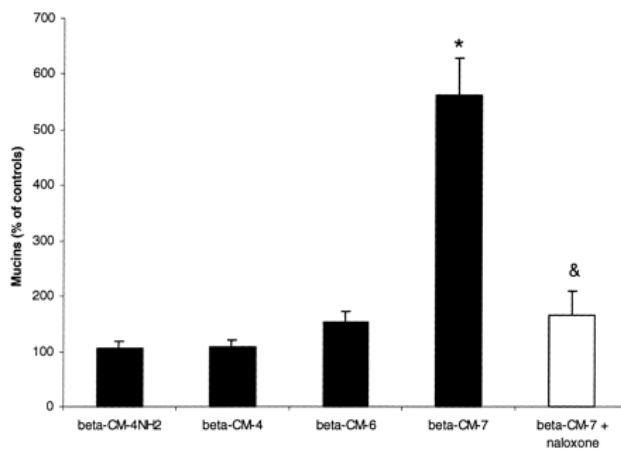
Gastrointestinal mucus secretion is at least partly regulated by opioid receptors; it is suppressed by opioid antagonists, such as naloxone,¹³ and increased by morphine.¹⁴ Gastrointestinal mucus serves as a protective barrier between the epithelium and the lumen, containing potentially harmful compounds and microorganisms, as well as a lubricant to help food passage.¹⁵ Studies have shown that dietary peptides, including BCM-7, stimulate mucus release via μ -opioid

receptors (Figure 2).^{4, 16, 17} Although the clinical relevance of this effect is not fully understood, excessive mucus secretion may interfere with commensal bacteria¹⁸ or alter gastrointestinal uptake of nutrients or drugs.¹⁹

Another component of the gut's immune system is the lamina propria lymphocytes. These cells play an important role in innate immunity and protection against pathogens in the intestinal lumen.^{20, 21} However, abnormal activity of the gut's immune system is implicated in the aetiology of gastrointestinal disorders, such as inflammatory bowel disease²¹ and food allergies.²² At least two studies have shown that BCM-7 alters lymphocyte proliferation *in vitro* through a pathway mediated by opiate receptors.^{23, 24} The first of these studies showed suppressive effects of BCM-7 on lymphocyte proliferation at all concentrations tested,²³ while the second study showed suppressive effects of low BCM-7 doses and stimulatory effects at higher doses.²⁴ Considering that both studies were performed *in vitro*, more studies are needed to confirm the physiological relevance of these immunomodulatory effects of BCM-7 in animals and humans.

Studies have also examined the possible mechanisms underlying the pro-inflammatory effects of BCM-7. UI Haq et al.²⁵ orally administered BCM-7 or BCM-5 to mice and revealed that both peptides increased the expression of inflammatory markers (myeloperoxidase, monocyte chemotactic protein-1, and interleukin-4) and immunoglobulins involved in the humoral immune response), enhanced leukocyte infiltration into intestinal villi, and increased expression of Toll-like receptors in the gut. The authors concluded that both peptides activate inflammatory responses through the Th₂ pathway.

Figure 2. BCM-7 significantly enhances mucus secretion in isolated rat jejunum. Reprinted from Trompette et al (2003).¹⁷



Effects of A1 and A2 β -casein on gastrointestinal function

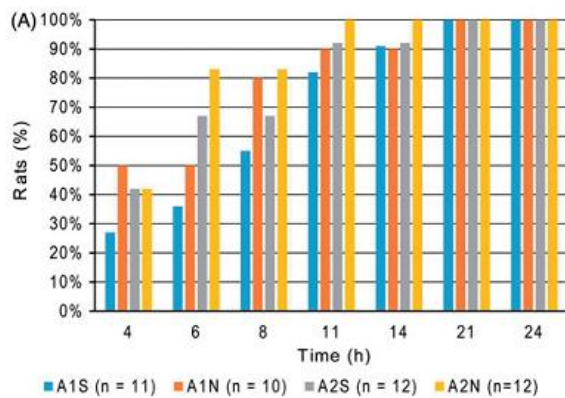
Several studies have examined the effects of A1 and A2 β -casein on gastrointestinal function. In one study, the authors fed Wistar rats milk-based diets in which the β -casein component was of the A1 or A2 type for 36 or 84 h.²⁶ Some rats in each group were co-administered naloxone to inhibit μ -opioid receptors. The authors measured gastrointestinal transit time, myeloperoxidase activity, and DPP-4 activity. Administration of the A1 diet slowed gastrointestinal transit and increased the colonic activities of myeloperoxidase and DPP-4 compared with the A2 diet (Figure 3). Co-administration of naloxone attenuated the effects of the A1 diet on gastrointestinal transit time and myeloperoxidase activity, but not DPP-4 activity, and had no effects on any of these variables in rats fed the A2 diet. The results of that study indicate that the A1 diet has direct effects on gastrointestinal function by slowing transit time and increasing the expression of myeloperoxidase and DPP-4. As myeloperoxidase is a marker of neutrophil activity, its increased expression might indicate pro-inflammatory effects of A1 β -casein. DPP-4 is an important digestive enzyme that is clinically relevant in terms of the treatment of type 2 diabetes because its substrates include glucagon-like peptide-1, which is involved in the regulation of insulin secretion and glucose metabolism, and because it is the pharmacological target of DPP-4 inhibitors.^{27, 28} Thus, consumption of diets containing A1 β -casein might have unwanted effects on glucose metabolism. Of note, the effects of A1 β -casein on gastrointestinal transit time and myeloperoxidase, but not DPP-4 activity, were attenuated by naloxone. These findings provide further support for a role of opioid signalling pathways in the effects of A1 β -casein.

Similar findings were reported by Ul Haq et al,²⁹ who found that A1-like variants of β -casein had pro-inflammatory effects in the gut via increasing the levels of inflammatory markers (myeloperoxidase, monocyte chemoattractant protein-1, and interleukin-4) and immunoglobulins, and enhancing leukocyte infiltration and Toll-like receptor expression. These effects were not observed in mice fed A2 β -casein.

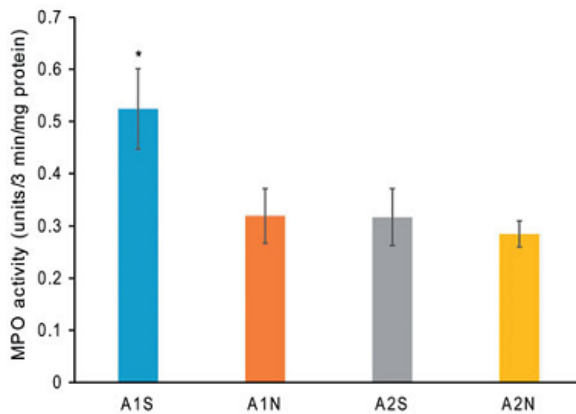
Taken together, the results of both studies highlight the pro-inflammatory effects of A1 β -casein, and suggest that A1 β -casein might contribute to a variety of clinical disorders, especially gastrointestinal disorders.

Figure 3. Effects of A1 and A2 β -casein diets on (a) gastrointestinal transit (measured as titanium dioxide recovery over 24 h), (b) myeloperoxidase activity in the jejunum, and (c) DPP-4 activity in the jejunum. Rats were fed a diet containing A1 or A2 β -casein and were treated with either saline (S) or naloxone (N). *P < 0.05 vs the other groups; **P < 0.01 vs. A2S. Reprinted from Barnett et al (2014).²⁶

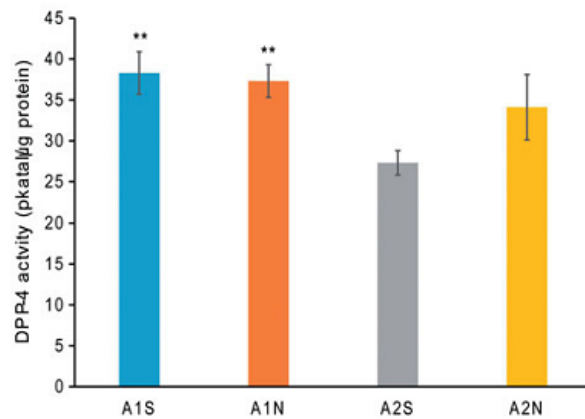
A



B



C



A1 protein avoidance could lessen gastrointestinal disturbances

The reports described above suggest that the consumption of products containing solely A2 β -casein and exclusion of those containing A1 β -casein could have some health benefits by maintaining the nutritional benefits associated with milk consumption while excluding the negative effects of A1 β -casein-derived BCM7 on gastrointestinal function.

One gastrointestinal disorder commonly associated with milk consumption is lactose intolerance. However, some patients may have food allergy rather than intolerance, or they may adversely react to other components of milk.³⁰ In this way, reducing or stopping the intake of A1 protein could provide a first step toward isolating the cause of the gastrointestinal disturbance. Indeed, recent media reports have highlighted this possibility in a child with apparent lactose intolerance who did not experience gastrointestinal symptoms after switching to products containing A2 protein, suggesting that this child experienced an immune response to a component of A1 protein.^{31, 32}

In a randomized, double-blind, crossover study in which subjects consumed A1 type milk or A2 type milk for 8 weeks each, the A1 type milk was associated with greater abdominal pain, bloating, and softer stools than was the A2 type milk.³³ These subjective assessments of gastrointestinal function were more pronounced in subjects with dietary intolerance, assessed in terms of elevated faecal calprotectin levels. These preliminary findings suggest that consumption of A2 β -casein could attenuate abdominal discomfort associated with the consumption of A1 β -casein, especially in dietary intolerant individuals.³³

Further large-scale, prospective, randomized controlled studies are needed to confirm the validity of avoiding A1 β -casein for avoiding gastrointestinal disturbances, but clinicians should be aware of the effects of BCM-7 and A1 β -casein on gastrointestinal motility, mucus secretion, inflammation, and immune function, and could support such a strategy in clinical practice.

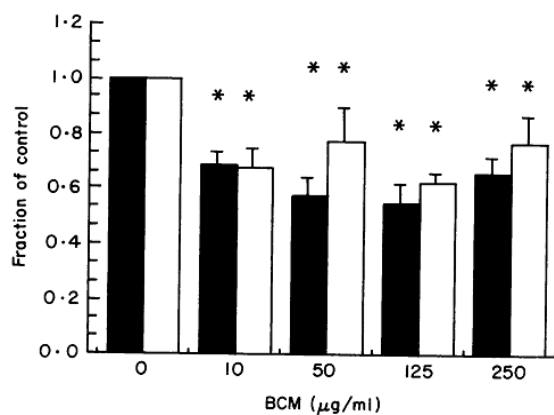
Immunomodulatory effects of exorphins

While the immunomodulatory effects of morphine are generally well established, the potential immunomodulatory effects of β -casein and its cleaved peptides were first identified in the 1980s.^{34, 35} Since then, it has become apparent that exorphins, including BCM-7, have immunomodulatory properties. For example, BCM-7 was reported to trigger histamine release from peripheral leukocytes³⁶ and to have secretagogue effects on peritoneal mast cells.³⁷

Studies have shown that BCM-7 alters lymphocyte proliferation in vitro through a pathway mediated by opiate receptors.^{23, 24} The first of these studies showed suppressive effects of BCM-7 on lymphocyte proliferation at all concentrations tested (Figure 4),²³ while the second study showed suppressive effects of low BCM-7 doses and stimulatory effects at higher doses.²⁴

Clinically, BCM-7 may also stimulate excessive histamine release, resulting in activation of immune responses^{36, 38}. Impaired immune function may also increase susceptibility to infection and other potentially severe diseases, as has been reported for morphine.³⁹ Additional studies are needed to establish the specific immunomodulatory effects of BCM-7 and related peptides, and to determine their clinical implications.

Figure 4. BCM-7 suppresses thymidine incorporation, as a marker of cell proliferation, in lamina propria lymphocytes from the colon (black bars) and ileum. *P < 0.05 vs control. Reprinted from Elitsur et al. (1991).²³



A1-derived BCM-7 and respiratory function

Opioid receptors, including μ -opioid receptors, are widely expressed in the central nervous system, including in sites associated with respiration control in the pons.⁷ Consequently, activation of these receptors by morphine and other opioids can induce respiratory depression, which can be reversed by antagonists, such as naloxone.⁷

Peptides derived from casein, including BCM-7, have been implicated in the aetiology of sudden infant death syndrome.⁴⁰ For example, Wasilewska et al. noted that infants with apparent life-threatening events had higher serum levels of BCM-7 after apnoea compared with healthy infants of the same age.⁴¹ One explanation for their findings was that the level of

dipeptidyl peptidase 4 activity, which degrades short peptides in blood,⁴² was reduced in these infants, resulting in abnormally high levels of BCM-7.⁴¹ Similar findings were reported for other BCMs and β -endorphins.⁴³⁻⁴⁵ Hedner and Hedner noted that BCMs can readily cross the blood–brain barrier in newborn rabbits and cause dose-related depressions of respiratory frequency and tidal volume.⁴⁶ They found that BCM-7 was equipotent to morphine, and its effects were reversed or prevented by naloxone, a μ -opioid receptor antagonist.

Further clinical and experimental studies are needed to evaluate the clinical relevance of BCM-7 in respiratory function and dysfunction in infants and in adults. Studies should also seek to confirm the association between BCM-7 and apparent life-threatening events in infants, and whether avoidance of some protein sources could reduce the incidence of such events.

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Disclosure

This evidence-based report and others in the same series were developed by the medical communications branch of Edanz Group Ltd (Hong Kong) to summarize key research findings associated with bovine A1/A2 β -casein consumption. The reports were commissioned by The a2 Milk Company Limited (Auckland, New Zealand).