A1 & A2 Beta-casein and gastrointestinal effects
Lactose intolerance
Lactose intolerance has been described as one of the most common intolerance responses to cows’ milk(9), with an estimated prevalence of around 10% in Caucasian Australians(22).

However, for a proportion of adults who report intolerance symptoms following milk intake, lactose intolerance is not the cause, as cases of perceived lactose intolerance are more common than its prevalence in adults(13-15). For instance, in a group of 406 randomly recruited men and women (mean age 27 years), 20.2% reported abdominal discomfort following dairy intake, but only 6.4% had lactose intolerance diagnosed by a physician(19).

Other natural components in milk may be responsible for stimulating some of the remaining gastrointestinal (GI) intolerance responses and indeed there is recent evidence to support that cows’ milk A1 beta-casein protein type may also be involved in some people(21).

A1 and A2 Beta-casein milk proteins
Beta-casein is a cows’ milk protein that makes up around 30% of the total protein contained in cows’ milk and may stimulate effects beyond nutrition, due to the release of biologically active peptides on digestion(21). Beta-casein may be present as one of two major genetic variants: A1 and A2(9). A2 is recognised as the original beta-casein in variant because it existed before a mutation caused the appearance of A1 beta-casein in some European herds a few thousand years ago(10, 11).

In Australia, regular cows’ milk contains a mix of A1 and A2 beta-casein types. In contrast, cows’ milk known as a2 Milk™ comes from cows with the genes to produce only the A2 beta-casein type (Figure 1).

Cows’ milk A1 beta-casein is different to cows’ milk A2 beta-casein and other mammalian beta-caseins. A1 beta-casein has a histidine at position 67 on the protein chain. Cows’ milk A2 beta-casein, human milk, goat milk, sheep milk and other species’ milk have a proline at their equivalent positions on their beta-casein protein chains, making them ‘A2 like’(12-14). Due to this amino acid variation, A1 beta-casein releases the bioactive opioid peptide beta-casomorphin-7 (BCM-7) upon normal enzymatic digestion(15-19) (Figure 2).

A1 beta-casein content in ordinary cows’ milk
In Australia, where dairy cows are of northern European ancestry, the relative proportions of the co-dominant A1 to A2 beta-casein genes in cows are typically 1:1, which then produce the same ratio of A1 to A2 beta-casein in their milk (Figure 1A). This tends to be lower in breeds from Southern Europe and this ratio depends on the specific breeding history of the dominant breeds. In Australia, most cows’ milk available commercially contains a mix of A1 and A2 beta-casein. The exception to this is milk produced by dairy cows genotyped and identified to carry only the un-mutated A2 beta-casein expressing gene on both sides of the chromosome (i.e. a2 Milk™). These dairy cows produce milk containing only the A2 beta-casein type and not the A1 (Figure 1B).

**Figure 1:** A1/A2 beta-casein gene carrying cow, which produces milk containing a 50:50 A1:A2 beta-casein mix. B) A2:A2 beta-casein gene carrying cow, which produces A2 beta-casein but no A1 beta-casein in her milk.

**Figure 2:** A1 and A2 proteins showing the amino acid difference at position 67, representing the release of BCM-7. Figure adapted from reference(20).

**Figure 3:** Beta-casein content and potential BCM-7 release per 250mL of milk. Figure adapted from reference(28).

A2 beta-casein releases much less and probably minimal amounts of BCM-7 under normal gut conditions(13-15). BCM-7 was characterised as a casein-derived opioid peptide more than three decades ago(21-24). BCM-7 is a mu-opioid receptor ligand(21, 24) and mu-opioid receptors are expressed widely throughout human physiology, including the gastrointestinal tract(25).
A1 Beta-casein and gastrointestinal effects

Two recent animal studies have investigated cows’ milk A1 versus A2 beta-casein proteins on GI effects directly\(^{29, 30}\). Feeding rodents milk containing A1 beta-casein resulted in significant delays in GI transit time and increased colonic activity of the inflammatory marker myeloperoxidase (MPO), compared to milk containing A2 beta-casein\(^{29}\).

Similarly, feeding mice a milk free basal diet supplemented with A1 relative to A2 beta-casein also resulted in significant MPO level increases (by 204%), whereas A2 beta-casein had no effect relative to controls\(^{30}\).

Cows’ milk protein\(^{31, 32}\) and more specifically the casein milk protein\(^{33, 34}\) have been shown previously to be associated with various effects on the GI tract, including the inhibition of motility\(^{34, 35}\), with evidence implicating exogenous opioids like BCM-7 as a mediator\(^{36–39}\).

In dogs, a comparison of casein and soy protein on various GI motility measures (e.g. force and contraction frequency) showed that casein reduced these parameters significantly and that pretreatment with naloxone (an opioid antagonist) blocked this effect\(^{35}\), suggesting a role for exogenous opioids like BCM-7. Such BCM-7 effects on GI motility are physiologically plausible, since BCM-7 is a mu-receptor ligand and mu-receptor activation is known to affect the mechanics of intestinal propulsion\(^{40}\).

a2 Milk™ may assist some people with milk mediated digestion symptoms

A human randomised crossover study comparing the effects of A1 versus A2 beta-casein milk proteins (A1 vs a2 Milk™) on GI outcomes has shown significant differences in stool consistency, with stools on A1 being overall looser\(^{37}\). For people with looser stools on the A1 milk, there was very strong evidence that this was associated with more abdominal pain (\(P<0.001\)). This relationship was absent when the same people consumed a2 Milk™. The difference between these two correlations was highly significant (\(r=0.40\), \(P=0.001\)), suggesting a role for exogenous opioids like BCM-7. These studies collectively suggest the softer stools observed by people consuming A1 compared with A2 beta-casein milk\(^{37}\) are caused by proinflammatory factors coupled with GI transit time effects.

Forty-one people were recruited into this double-blinded, randomised crossover study. Most participants at study entry considered themselves not to have problems digesting ordinary milk. Participants underwent a 2-week dairy washout (rice milk replaced all dairy), followed by two weeks of 750mL/milk/day containing beta-casein of either the A1 or A2 type, before undergoing a second washout followed by a final two weeks of the alternative type milk.

In addition to the above results, this study identified that while on the A1 milk, higher gut inflammation (faecal calprotectin) correlated with higher abdominal pain (\(r=0.46\), \(P=0.005\)) and higher bloating (\(r=0.36\), \(P=0.03\)) scores but that on the a2 Milk™ and in the same people, these relationships were absent. Again, the difference in the correlation measures was significant for: 1) gut inflammation and abdominal pain (A1, 0.46 vs A2, 0.03; \(P=0.02\)); and 2) gut inflammation and bloating (A1, 0.36 vs A2, −0.02; \(P=0.05\)).

A sub-group analysis of study participants with self-reported intolerance to ordinary A1 containing milk (n=8) showed further that the A1 milk resulted in more GI symptoms than the a2 Milk™ (Figure 5), and while it was not possible to demonstrate statistically significant differences, the magnitude of these differences between A1 versus a2 Milk™ may be clinically significant. Future studies examining this in different population groups with GI conditions, such as irritable bowel syndrome, are needed.

Given the previous animal research that shows A1 beta-casein feeding delays gut transit via an opioid pathway\(^{29}\) and that A1 compared with A2 beta-casein feeding significantly increases gut inflammation as evidenced by MPO levels\(^{29, 30}\), these studies collectively suggest the softer stools observed by people consuming A1 compared with A2 beta-casein milk\(^{37}\) are caused by proinflammatory factors coupled with GI transit time effects.

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A1 & A2 Beta-casein and gastrointestinal effects… where do lactose intolerance and a2 Milk™ fit in?

While further clinical trials are needed to confirm these preliminary results in humans relating to GI response differences between A1 and A2 beta-casein proteins in milk, the following has been established:

- Digestion of A1 beta-casein yields BCM-7, while A2 beta-casein does not or if it does, at a very low rate [22].
- BCM-7 is an exogenous opioid that can bind mu-opioid receptors expressed in cells throughout the body, including those found in digestive tissue [23, 41].

**References**


