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# A1 and A2 milk: a scientific overview



## EDITORIAL

In Ireland, 7% of consumers believe that dairy 'is difficult to digest' and this is often attributed to lactose intolerance. However lactose intolerance is present in just 4-5% of the Irish population. For these individuals, lactose-free milk is a suitable option and its scientific validity is well established.

As some intolerance may be unrelated to lactose, A2 milk has been proposed as a potential solution for individuals who experience digestive discomfort following the consumption of conventional cow's milk. It is speculated that these individuals may have an inflammatory reaction to compounds from the casein component of milk. However, the science in this area is both sparse and conflicting.

This edition of *DN Forum* explores the A1/A2 milk hypothesis, reviewing the latest science in the area. We hope you enjoy this edition and look forward to any feedback or comments you wish to share: [nutrition@ndc.ie](mailto:nutrition@ndc.ie)

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## Summary points

- The market value of A2 milk is growing globally. Many consumers see it as a suitable option for those who experience digestive discomfort following the consumption of conventional milk.
- Caseins are the most abundant proteins in cow's milk and consist of four main types ( $\alpha_{s1}$ -,  $\alpha_{s2}$ -,  $\beta$ - and  $\kappa$ -caseins). For the  $\beta$ -casein fraction, A1 and A2 variants are the most well-known. Conventional cow's milk usually contains a mix of both variants. A2 milk has the same composition as regular cow's milk, but simply contains only the A2 variant of  $\beta$ -casein.
- The small difference in amino acid sequence between A1 and A2 milk impacts how the proteins are broken down during digestion. The A1 variant releases a much greater amount of  $\beta$ -casomorphin 7 (BCM-7), a seven-amino acid peptide. BCM-7 has been shown to have opioid like properties and it has been speculated that it can initiate inflammation.
- Assessment by Food Safety Authorities concluded that there are no food safety risks relating to A1 milk and insufficient evidence linking it to any non-communicable diseases. There is suggestive evidence, however, that A2 milk could be beneficial in alleviating symptoms of gastrointestinal distress in a certain demographic of individuals. Nonetheless, the mechanisms for these interactions and criteria to identify consumers that would benefit from A2 milk are still poorly understood. Therefore, evidence to support promotion of A2 milk is currently insufficient.

# An overview of the A1/A2 milk hypothesis

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Dr Tom F. O'Callaghan

## Introduction

Milk and dairy products are an excellent source of proteins, lipids and a wide range of essential vitamins and minerals<sup>(1)</sup>. A2 milk is produced by cows with a specific genetic trait. In essence, it is the same as regular cow's milk, apart from this genetic difference, which is responsible for the expression of the  $\beta$ -casein protein. The marketing of A2 milk suggests that it may be a preferable option to conventional milk, for those wishing to ease issues relating to digestive discomfort and inflammatory processes in the body. The concept has been the topic of significant scientific debate and this review aims to provide an up-to-date synopsis of the research. Protein typically accounts for 3.2-3.5% of cow's milk composition; within this fraction there are different sub-groups of proteins, with casein and whey proteins predominating. Casein and whey proteins are present at a ratio of 80:20. The casein family of proteins can be further sub-classified into four main fractions  $\alpha_{s1}$ -,  $\alpha_{s2}$ -,  $\beta$ - and  $\kappa$ -caseins<sup>(2)</sup>, with each of these casein fractions having several different DNA variants (Figure 1).

The protein composition of milk can be affected by a variety of factors such as animal feed, health status, breed, stage of lactation and age. However, the variation of these casein proteins (i.e.  $\beta$ -casein A1 or A2), is solely under the control of animal genetics.  $\beta$ -casein is the second most abundant casein in cow's milk encoded by the CSN2 gene on chromosome 6, and consists of a 209 amino acid single polypeptide chain<sup>(3)</sup>. It can be present in a number of different variants ranging from A1, A2, A3, B, C, D, E, F, G, H1, H2, and I<sup>(4)</sup>, with the most prevalent forms being A1, A2 and B<sup>(3,5)</sup>. These variants differ in their distribution of amino acids in the primary sequence of  $\beta$ -casein. In terms of A1 and A2 milk, these differ in the amino acid sequence at position 67; where A1 milk has a histamine (His) residue, A2 milk has a proline (Pro) residue (Figure 2). It is worth noting that similar to A1 milk, the B, F and G alleles of  $\beta$ -casein also have a His residue at position 67 and a similar initial amino acid sequence<sup>(3,6)</sup>. The F and G alleles are far less frequent. As such, these less frequent alleles receive little attention while the main focus tends to be placed on the differences between the more frequent A1 and A2 variants. For simplicity, these are generally termed A1 or A2 milk.

Genotyping of the main dairy herd in Ireland suggests that the population is composed of approximately 15% A1A1 cows, 40% A2A2 cows and 45% A1A2 cows. Theoretically, A2A2 cows could be isolated to produce A2-only milk but currently this is not done commercially. Therefore, most conventional milk on the supermarket shelf in Ireland is a mix of A1 and A2 type milks. While production of A2-only milk is not as common in Europe, it has considerable market value in other parts of the world, primarily Australia and New Zealand, with growing markets in the USA and Asia-Pacific. As the market for A2 milk is growing globally and Irish consumers are becoming more aware of the product, it is important to assess the latest science in the area.

## Biological action during metabolism of A1 and A2 proteins

During digestion and milk processing (such as fermentation), milk proteins are hydrolysed releasing bioactive peptides, which have a number of biological properties. These include opioid, blood pressure lowering, antimicrobial and immunomodulatory effects, associated with the cardiovascular, nervous, digestive and immune systems<sup>(7,8,9,10)</sup>.

When A1 milk undergoes digestion, the parent  $\beta$ -casein protein is cleaved at position 67, releasing  $\beta$ -casomorphin (BCM) peptides,

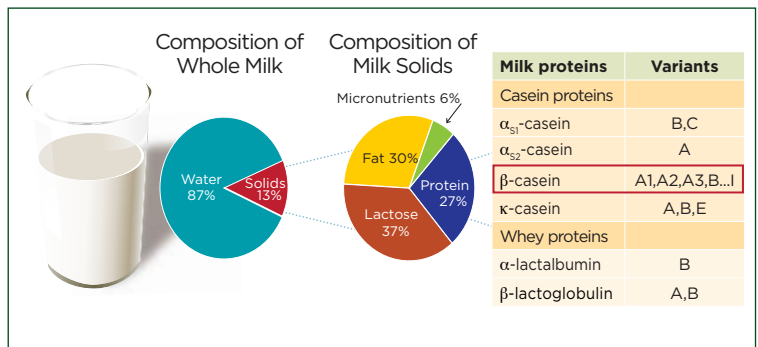


Figure 1: Variants of casein and whey proteins (including A1 and A2 as variants of  $\beta$ -casein).

including  $\beta$ -casomorphin 7 (BCM-7)<sup>(11)</sup>. The BCM-7 peptide has been shown to have opioid-like properties. In A2 milk, the substitution of Pro at position 67 in the sequence prevents this hydrolysis and thus does not, or greatly reduces, the release of BCM-7.

Several studies using simulated digestion methodologies have reported that BCM-7 can be released from both the A1 and A2 variants, but it has been demonstrated that the A1 variant is more potent, producing ~3 fold more BCM-7 than that of A2<sup>(12,13)</sup>. The notion that digestion of milk protein results in the release of peptides with opioid like activities is not novel, as a number of opioid peptides have been reported from different protein fractions, including human milk, goat milk, sheep milk, buffalo milk, cereals and vegetables<sup>(14)</sup>. However, some early reports speculated that BCM-7 and related compounds could have adverse effects and may contribute to development of juvenile diabetes type-1, ischemic heart disease and digestive discomfort. Autism and schizophrenia have also been tentatively associated with A1 milk consumption, but this has been dismissed as there is no convincing evidence at present for such a relationship. One of the notable publications on the topic of A1 milk was a book released in 2007, titled 'The Devil in the Milk'<sup>(15)</sup>. Although unconfirmed, the hypothesis surrounding A1 milk has gained attention and led to several patents around the production and testing of A2 milk, as well as the launch of commercially available A2 milk products.

## Hypothesis testing of A1 and A2 milk

The debate between advantages and disadvantages of A1 and A2 milk has been the topic of scientific discussion and research for almost three decades. While several reviews have refuted claims about A2 milk<sup>(16)</sup>, these too have been challenged<sup>(17,18)</sup>. The topic has also been investigated by national food authorities to examine whether any evidence warranted a change in policy or further risk assessment relating to BCM-7. These include a report to the New Zealand Food Safety Authority in 2004<sup>(19)</sup> and a scientific report to the European Food Safety Authority (EFSA) in 2009<sup>(14)</sup>. Both reports concluded that evidence to suggest A1 milk could be harmful was insufficient and did not warrant government agencies to take any public health actions. Therefore, no changes were made to dietary recommendations, labelling requirements or the breeding approach of national herds<sup>(19)</sup>. The author of the New Zealand report did however highlight a need "to monitor the health claims being made for A2 milk to ensure they comply with existing regulations". The authors of the EFSA report concluded that "based on existing evidence a cause-effect relationship between oral intake of BCM-7 or related peptides and aetiology or course of any suggested non-communicable diseases cannot be established".

Since the publication of these reports, further research has been conducted, with some of this summarised by Summer *et al.*<sup>(6)</sup>. On review of the literature of the last decade, a small number studies have associated A1 milk with an increased inflammatory response and gut discomfort<sup>(6)</sup>. However, links with worsening of non-transmissible diseases still lack evidence<sup>(6)</sup>.

### Research on A1 milk and gastrointestinal distress

The European Food Safety Authority<sup>(14)</sup> acknowledged that bioactive peptides (including BCMs) released from food during digestion, can have an effect on gastrointestinal function through regulation of mucosal processes and release of gastrointestinal hormones. The report also acknowledged that BCMs can interact with endogenous opioid systems in the gastrointestinal wall, influence postprandial metabolism and prolong gastrointestinal transit time. More recent human studies have demonstrated adverse digestive effects in humans from A1 milk compared to A2 (with certain limitations), such as worsening of gastrointestinal transit time, abdominal pain, stool consistency and increases in the levels of some inflammatory markers<sup>(6)</sup>.

In a randomised, double-blind crossover trial with a 14-day treatment period, Jianqin *et al.*<sup>(20)</sup> examined the impact of A1/A2 milk versus A2 only milk in 45 Han Chinese subjects, with self-reported intolerance to commercial milk. The study reported that consumption of conventional milk (A1/A2) resulted in increased post dairy digestive discomfort symptoms, higher levels of inflammation related biomarkers, longer gastrointestinal transit times and lower levels of total faecal short chain fatty acids. The authors concluded that some symptoms of lactose intolerance may stem from inflammation triggered by A1 milk. Study limitations were acknowledged, however, including that the study duration may have been too short to elicit changes in some biomarkers or local inflammation. Also the 'smart pill' used to evaluate stomach and small bowel inflammation was not used at baseline; therefore, it was not possible to determine whether A2 milk genuinely influenced gastrointestinal transit time<sup>(20)</sup>.

Another randomised double-blind crossover trial in China, examined the impact of conventional milk (A1/A2) versus A2 milk in 600 subjects with self-reported lactose intolerance and digestive discomfort<sup>(21)</sup>. The study reported significantly reduced gastrointestinal symptoms in both lactose absorbers and lactose malabsorbers, following consumption of A2 milk. The authors stated that results should be interpreted with care given certain limitations, but concluded that adverse gastrointestinal symptoms in some individuals could be related to the presence of A1 milk protein rather than lactose. On review Summer *et al.*<sup>(6)</sup> found that the results did not clearly prove a major advantage of A2 milk with only slight improvements in gastrointestinal symptoms compared with A1 milk.

Another study, in Chinese preschool children, reported that conventional milk compared to A2 milk exacerbated the symptoms of digestive discomfort associated with lactose intolerance<sup>(22)</sup>. Certain limitations included shortened intervention period and potential variance in the scales used to assess baseline gastrointestinal symptoms due to self-reporting.

The ability of BCMs to modulate water and electrolyte absorption in the small intestine is a hypothesized mode of action for their anti-diarrheal action. Crowley *et al.*<sup>(23)</sup> however examined the impact of dairy protein on chronic functional constipation in children. While the study did demonstrate a relationship between chronic functional constipation and consumption of cow's milk, no significant effect between consumption of A1 or A2 milk protein was reported. Brooke-Taylor *et al.*<sup>(24)</sup> conducted a systematic review of 39 studies with a specific gastrointestinal focus to examine effects of A1 versus A2  $\beta$ -casein. The authors found that A2  $\beta$ -casein had favourable gastrointestinal effects in rodents and humans, compared to A1  $\beta$ -casein. Furthermore, the authors acknowledged the likelihood that sensitivity to A1 milk may vary both across and within populations<sup>(24)</sup>. Küllenberg de Gaudry *et al.*<sup>(25)</sup>, on systematic review of the topic, examined 15 randomised controlled trials, two case-control studies and eight

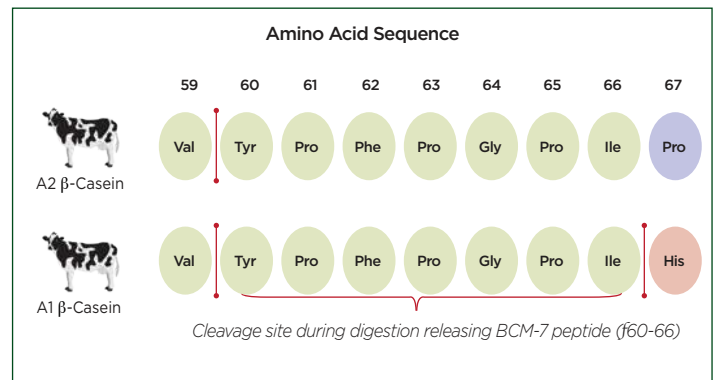


Figure 2:  $\beta$ -casein sequence with different amino acid sequences between A2 and A1 variants (highlighted fragments 59 - 67).

ecological studies. The authors concluded that available evidence from clinical trials and epidemiological studies published prior to October 2017 provides moderate certainty for adverse digestive health effects of A1  $\beta$ -casein compared with A2  $\beta$ -casein but low or very low certainty for other health effects. While research to date would suggest A2 milk could be beneficial in alleviating symptoms of gastrointestinal distress in a certain demographic of at-risk individuals, both the mechanisms for these interactions and the criteria to identify subjects that would benefit from A2 milk are still poorly understood. Further robust and independent research is required before concrete conclusions can be formed.

### Irish Research

Teagasc and VistaMilk (a Science Foundation Ireland Research Centre, [www.vistamilk.ie](http://www.vistamilk.ie)) are currently engaged in research on the impacts of animal genotype on the production and processability of milk. In 2019, a VistaMilk Masterclass with world leading experts was dedicated to the topic. Given the increased demand for A2 milk globally, more robust and independent research is needed to fully evaluate its effects in humans and understand the implications of converting herds to A2, should the need arise. Such research will also encompass effects of the genotype on milk production, yield, processability and other traits. Furthermore, a robust method for testing of A2 milk is required to confirm that milk marketed as such is, in fact, A2. This method should not just rule out A1 milk, but must also be able to confirm a product is not of the B, F or G allele, which could also result in the release of BCM-7 similar to A1 milk.

### Conclusion

While the existing research is not yet convincing, sales of A2 milk in certain regions of the world have risen at a significantly premium price compared to conventional milk (~+39% cost). As such the A2 Milk Company has become one of the most valuable companies on the New Zealand stock exchange<sup>(26)</sup>. While some evidence suggests a link between A1 milk consumption and gastrointestinal discomfort, it is likely that these effects pertain to a particular demographic of consumers. The exact mechanisms for these effects are still unclear. It is also worth noting that, in essence, the focus should not remain solely on A1 or A2 milk, as there are several variants that are capable of releasing the peptide of interest, BCM-7.

While some individuals may choose to reduce or remove A1 milk from their diet as a precautionary measure, they should do so knowing there is substantial uncertainty about the evidence to support such an approach.

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## New Resource

To address a range of dairy queries, the National Dairy Council have incorporated an A-Z of commonly asked questions on their website: [www.ndc.ie/dairy-myths/dairy-a-z/](http://www.ndc.ie/dairy-myths/dairy-a-z/).

It includes over 200 questions with concise answers on topics such as Health; Nutrition; Environment & Sustainability; Quality & Safety; Animal Welfare; and Dairy Products.



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Mission: To deliver real and unique value to Irish dairy farmers by protecting and promoting the image, quality, taste and nutritional credentials of Irish dairy produce to a wide variety of audiences in a clearly defined, focused and effective manner.

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Mission: To leverage the world-class capabilities of the Irish academic partners, with the market expertise of the industry partners, into a pipeline of innovative, nutritional functional ingredients/products for the global food industry.

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