Milk Proteins and Human Health: A1 versus A2 beta-casein proteins

Bonnie Johnson, MS, RDN
Vice President, Scientific Affairs
The a2 Milk Company--USA

Elizabeth Shaw, MS, RDN, CLT
Shaw’s Simple Swaps
Disclosures

• Bonnie Johnson is an employee of The a2 Milk Company
• Elizabeth Shaw is a consultant for The a2 Milk Company
Learning Objectives

To provide an overview on the difference between A1 and A2 beta-casein proteins and discuss the scientific evidence around beta-casein protein variants and potential impact human health.

After participating in this program, dietitians will be able to:

• Differentiate between A1 and A2 beta-casein proteins.
• List mammalian milk containing A1 and / or A2 beta-casein genetic variants.
• Evaluate the evidence for digestive differences between A1 and A2 beta-casein protein variants.
Introduction

• A1 beta-casein is relevant to digestive function and broader aspects of human health.

• In 2014, an important human cross-over A1 versus A2 beta-casein milk protein study provided preliminary evidence that these protein variants stimulate differences in digestion responses.

• Over the last three years, more studies have reported differences in gastrointestinal outcomes with feeding A1 versus A2 beta-casein.
Composition of milk

Milk Proteins & Human Health
Variants of β-casein

Originally all cows produced milk containing only A2 protein type.

Genetic mutation occurred in European herds via migration and modern farming practices.

All ordinary milk is a mix of A1 and A2 proteins.

a2MC milk naturally contains only the A2 protein and free from A1 protein.
A1 β-casein-free milk

- Goats, sheep, water buffalo and human breast milk contain A2-type beta casein protein.

- Due to a genetic mutation, cows can produce milk with three variations of β-casein:
  - A1/A1
  - A1/A2
  - A2/A2

- Most dairy operations pool all of this milk together so conventional milk is a mix of A1/A2 β-casein proteins.
- It is possible to identify cows that produce A2/A2 through a simple genetic test. Milk produced by these cows is generally considered A1 protein-free.
The difference between A1 and A2 \( \beta \)-casein families

• The only difference in the **209 amino acid chain of beta-casein** is a single amino acid at position 67 (proline in A2 instead of a histidine A1)

• However, this has a profound effect on the way the protein digests.

(Kaminski et al., 2007)
Peptides released on digestion

Beta-casomorphin-7 (BCM-7) only released from A1

(Jinsmaa, et al. 1999; Ul Haq et al. 2015; Wada Y, et al. 2015)
Some known effects of A1 β-casein protein & bovine BCM-7

• Under normal digestive conditions in the human gut, BCM-7 is released from A1 β-casein but not A2 β-casein (De Noni, 2008; Boutrou et al. 2013)
• BCM-7 has opioid characteristics and this is the reason for the ‘morphin’ in the name (Henschen, A. 1979)
• BCM-7 cause inflammation in all tissues.
• Most compelling and comprehensive research shows impact of BCM-7 in the gastrointestinal tract. (Ul Haq et al. 2014; Barnett et al. 2014)

Myeloperoxidase activity in the ileum. MPO is a marker of inflammation

Interleukin 4 in the ileum

Different letters indicate (p<0.05)
1st Human Clinical Trial (Curtin University, Australia)

- 41 participants, all of whom spent two weeks on each of an A1 beta-casein and an A2 beta-casein milk diet, with the order randomly assigned, with a two week washout period both before and between the treatments
- 31 of the participants were normal milk drinkers and considered themselves to have no intolerance to milk.
- 10 did consider themselves to have an intolerance issue but most of these still normally consumed milk products.
- Five withdrew during the trial (four on the A1 arm)

(Ho et al. 2014)
Curtain University Trial

Methods:
• All received 100% A1 β-casein milk or A1 β-casein-free Milk.
• VAS intolerance measures:
  • abdominal pain
  • bloating
  • voiding difficulty
  • stool consistency
• Fecal calprotectin values.

Results:
• 100% A1 β-casein milk increased stool consistency. No effect with A1-free milk.
• Positive association between abdominal pain and A1 β-casein. No effect with A1-free milk in any participant.
• Fecal calprotectin values correlated highly with VAS measures on A1 β-casein. Weakly on A1-free milk.

Ho et al. (2014), Eur Jour Clin Nutr
Human cross-over A1 versus A2 β-casein milk study - pain and looser stools on A1 but NOT on A2

- There was a significant **positive** association between abdominal pain and stool consistency on the A1 diet ($r = 0.520, p=0.001$), **but not the A2 diet** ($r = -0.13, p=0.43$). The difference between these two correlations (0.52 versus -0.13) was highly significant ($p<0.001$).

- **Interpretation/explanation:** Slower GITT caused by A1 β-casein containing milk increased opportunities for fermentable gut contents to undergo fermentation, which did not occur with the A2 beta-casein containing milk diet.

(Ho, et al. 2014)
2016 Human Clinical Trail (Fudan University, China)

- 45 participants, all of whom spent two weeks on each of a conventional milk and an A1 protein-free milk (A2) intervention, with the order randomly assigned, with a two week washout period both before and between the treatments.

- All participants self-reported post-dairy digestive discomfort (PD3); 23 participants tested positive for lactose intolerance based on urinary galactose test.

- 10 subjects reported adverse events (episodes of diarrhea); 5 were considered related to consumption of conventional milk; 3 were considered related to A1 protein-free milk; 2 were considered unrelated to the intervention. None were significant enough for withdrawal from the study.
Fudan University study design

- A1/A2 milk refers to milk containing both A1 and A2 beta-casein (regular cows’ milk)
- A2 milk refers to milk free-from A1 beta-casein
Results: VAS Gastrointestinal symptoms

- Significant change from baseline was observed in both sequence groups on the VAS score for bloating, flutulence and borborygmus during consumption of conventional milk.

- The VAS score for all gastrointestinal symptoms was the same as baseline values and washout period (p<0.05) for both sequence groups during the consumption of A2 milk.
Results: Analysis in subjects with lactose intolerance

The magnitude of the increase in gastrointestinal symptoms scores following the consumption of conventional milk is greater in lactose intolerant subjects than lactose tolerant subjects.
Results: Stool frequency & consistency

Consumption of conventional milk was associated with increase in both stool frequency and Bristol Stool Scale scores compared with baseline.

Consumption of A2 milk was not associated with changes in either variable.
Results: Gastrointestinal inflammation

Change of small-bowel inflammation was significantly different between subjects under different intervention sequence (p=0.042), while no sequence difference was observed for change of stomach inflammation during the study.

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Evaluation</th>
<th>Sequence A1-A2 (n=22)</th>
<th>Sequence A2-A1 (n=18)</th>
<th>Fisher Exact Test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach Inflammation</td>
<td>Improved</td>
<td>5 (22.7%)</td>
<td>2 (11.1%)</td>
<td>0.427</td>
</tr>
<tr>
<td></td>
<td>No change</td>
<td>17 (77.3%)</td>
<td>16 (88.9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Worsen</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Small-bowel Inflammation</td>
<td>Improved</td>
<td>8 (36.4%)</td>
<td>2 (11.1%)</td>
<td>0.042 *</td>
</tr>
<tr>
<td></td>
<td>No change</td>
<td>14 (63.6%)</td>
<td>15 (83.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Worsen</td>
<td>0 (0.0%)</td>
<td>1 (5.6%)</td>
<td></td>
</tr>
</tbody>
</table>
Summary
Consumption of conventional milk in milk intolerant participants was associated with:

- Greater gastrointestinal symptoms & longer transit time
- Higher concentration of inflammation-related biomarkers

Consumption of A2milk was comparable to washout (rice milk) with all the measures and tolerated in those with confirmed Lactose Intolerance.

Consumption of conventional milk (containing A1 β-casein) is associated with greater gastrointestinal symptoms in Lactose Intolerant subjects than in those tolerant of lactose.

Interpretation/explanation
In sensitive subjects, A1 β-casein protein causes inflammation high in the small bowel (duodenum) which may decrease lactase expression lower in the small bowel (ileum) and manifest as Lactose Intolerance (lactase deficiency + symptoms).

(Jianqin, 2016)
β-casein involvement in gene expression

Milk Proteins & Human Health

Neuronal or Gut Epithelial cell

Food-derived opioid peptides inhibit cysteine uptake with redox and epigenetic consequences

Malen S Trivedi, Jayot B Shah, Sara Al-Mughairi, Katherine W Hodgson, Benjamin Simms, Geert A Tirokens, Wim Van Cremmer, and Richard C Dehn

Department of Pharmaceutical Sciences, Northeastern University, Boston, MA, USA

Department of Mathematical Modelling, Statistics and Bioinformatics, Faculty of Biosciences Engineering, Ghent University, Ghent, Belgium

Epigenetic Regulation of
Gene Expression

DNA Methylation

OPIATES

Mu Opiate Receptor

Cysteine

EAAT3

Cysteine

GSH / GSSG

Redox Status

Methylation Status

SAM / SAH
Snapshot of the science

- The science is clear that under normal digestion A1 beta-casein releases BCM-7 whereas A2 does not.
- The science is clear that BCM-7 is a mu-opioid receptor agonist.
- The science is clear that BCM-7 has physiological effects when fed to various animal species including humans.
- The mechanism of BCM-7 involvement in gene expression is clear.
- Additional gold standard trials are needed to substantiate claims of the digestive benefits of A1 protein-free milk in the US population in general, as well, as disease specific subsets of the population.
The a2 Milk Company--USA

• The a2 Milk Company first launched A1 protein-free milk in Australia/New Zealand in 2006. Since then, it has become the highest selling milk brand in that region.

• From cows that naturally only produce A1 protein-free milk.

• Proprietary quality assurance steps in place at the processor level to guarantee a2 Milk® brand is A1 protein-free.

• a2 Milk® launched in the US in April 2015 in California. Distribution is growing with expected national penetration by mid-2018.

• Ta2MC is investing millions of dollars in collaborative research with leading US institutions like the Pennington Biomedical Research Center, Purdue University and Baylor College of Medicine.


