

Bovine Beta Casein Variants and Digestion

26 September 2017

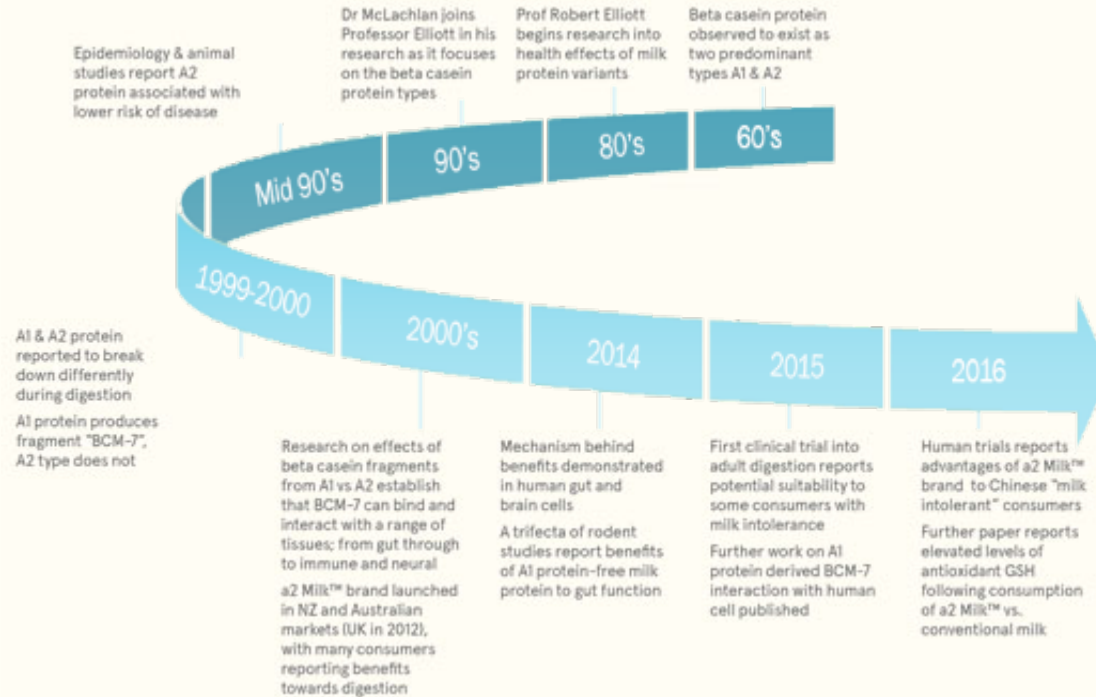
Host Institution



Outline

- Research Timeline
- Beta Casein Variants
- Review of Studies on Beta Casein and Digestive Function
- Recent Research
- The Future

Research Timeline

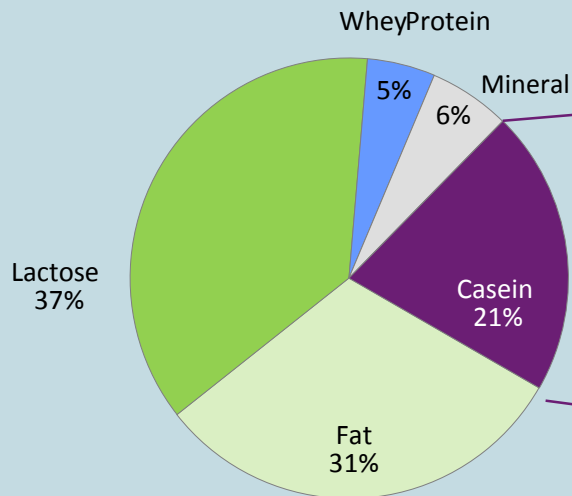


¹ Reflects calendar years

² Beta casomorphin-7

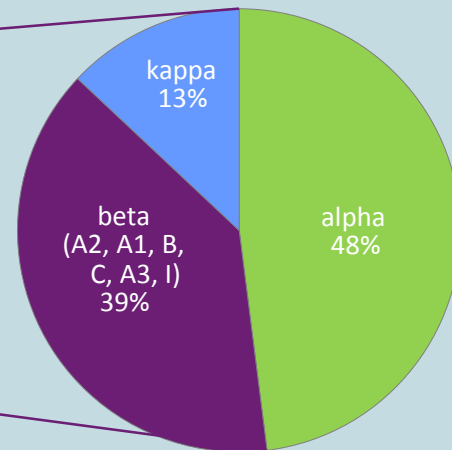
Bovine Beta Casein ~2.5 grams per serve

Composition of Solids in Milk



Conventional A1 containing milk and the a2 Milk™ brand have the same general composition

Composition of Milk Casein



a2 Milk™ brand contains only the A2 type of the beta-casein protein

A2 Beta Casein: The Original Variant

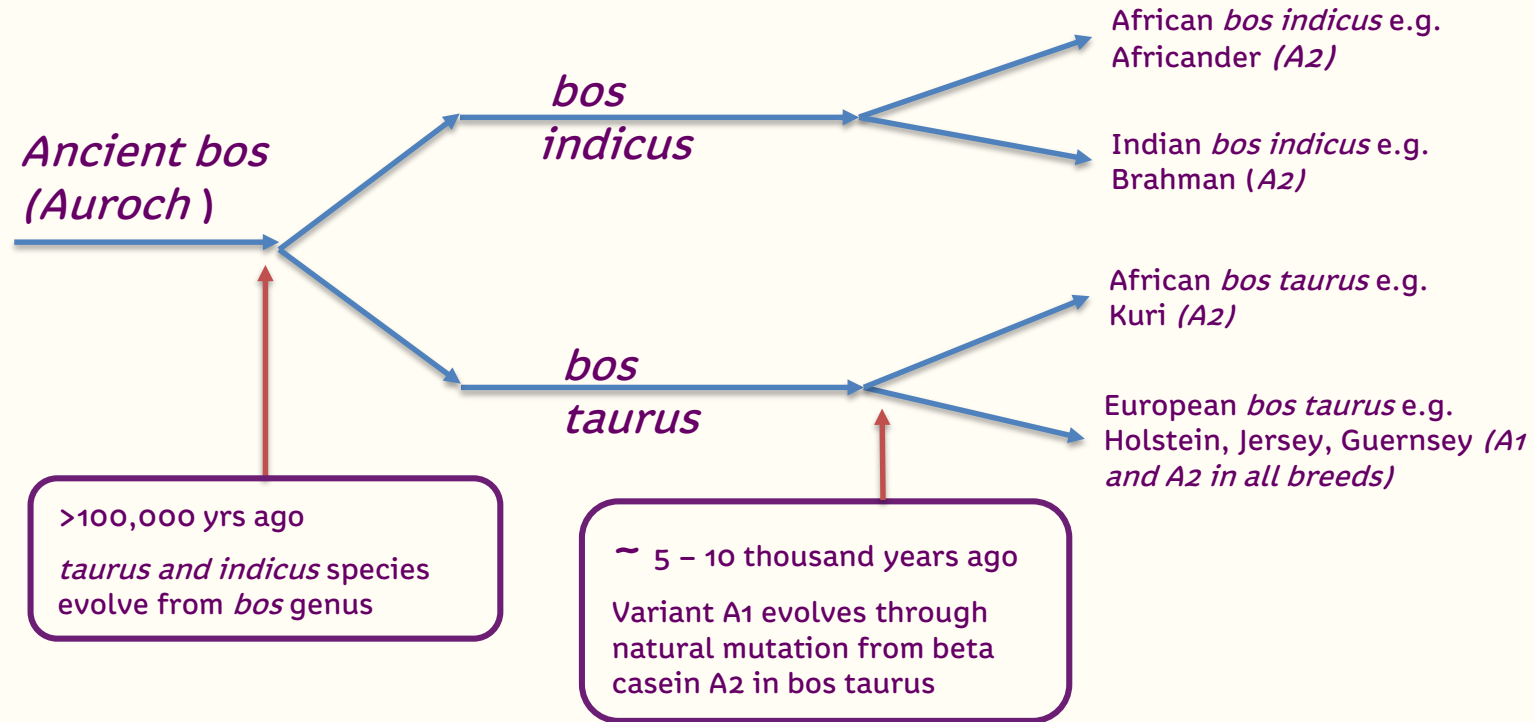


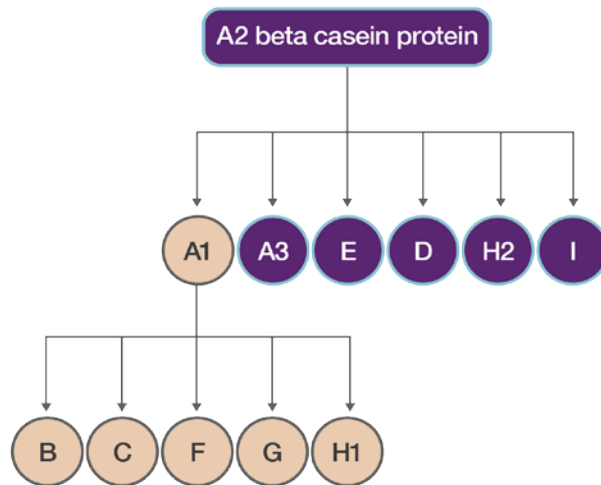
Figure adapted from information reported in:

1. *Advanced Dairy Chemistry: Volume 1 - Proteins*, Second Edition, edited by P.F. Fox and P. L. H. McSweeney pp 424 & 430
2. *Annali della Facoltà di Medicina Veterinaria*, Vol. XIX, 1999. Università degli Studi di Parma
3. Daniel G. Bradley, Ronan T. Loftus, Patrick Cunningham, David E. MacHugh (1998) *Evolutionary Anthropology* V6;3 pp79-86

Bovine Beta Casein Variants

- Originally all domesticated cows produced milk containing only the A2 type of beta casein
- Owing to natural genetic mutation, a variant of the A2 protein appeared; termed A1 protein, differs very slightly in composition from the original A2 protein
- Both types have since given rise to a number of minor related “sub-variants”, such as those termed A3, B and C
- Beta casein variants can be divided into either “A1 type” or “A2 type” based on their digestion
- A1 and A2 variants are the primary types of beta-casein
- Variants A3, B and I are increasing in frequency

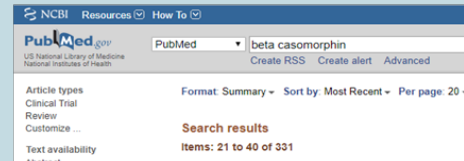
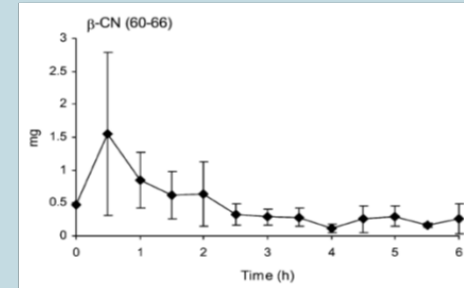
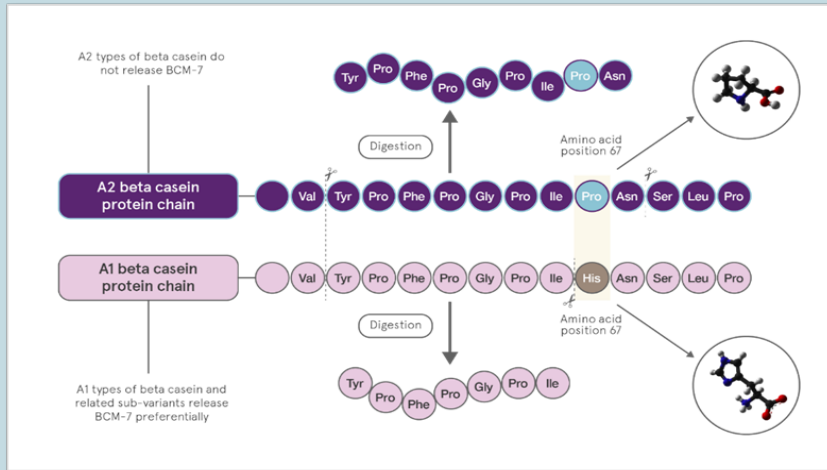
Evolution of the beta caseins in cow's milk from the original A2 type



A1 and A2 variants are the primary types of beta casein in a2 Milk™ branded milk which contains only the A2 type of the beta casein protein

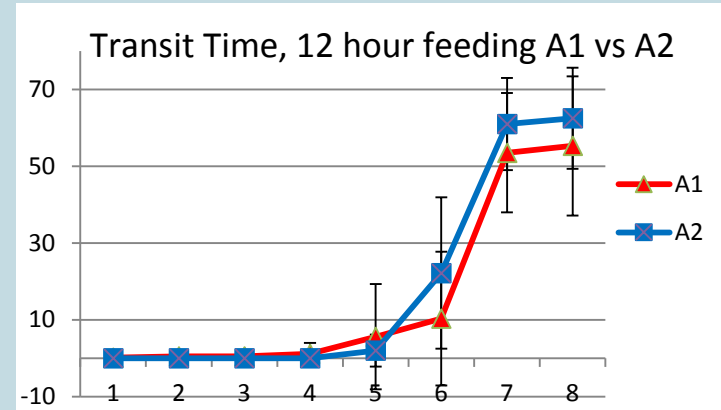
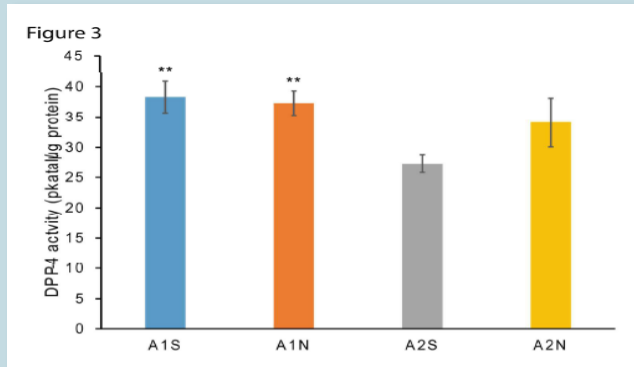
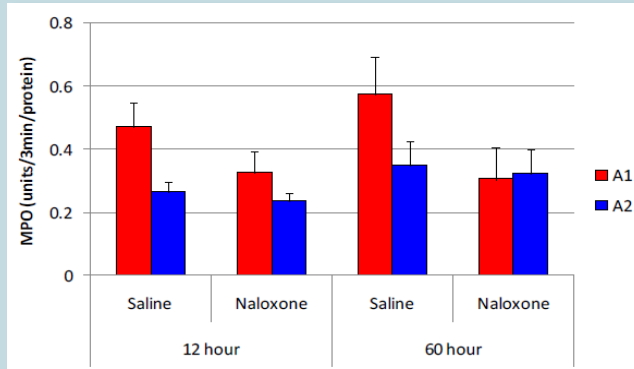
Digestion of Beta Casein Variants

- It is accepted that BCM-7 is produced from the incomplete digestion of the A1 but not A2 types of beta casein
- BCM-7 is an established exorphin, or peptide that binds and 'activates' opiate receptors expressed by cells and tissues throughout the body. (EFSA 2009)
- BCM-7 is produced at physiological levels in healthy adult human small intestine (Boutrou et al, 2013)



- Searching scientific or medical databases will throw up hundreds of published studies on BCM-7 or beta casomorphin

Barnett *et al* (2014)

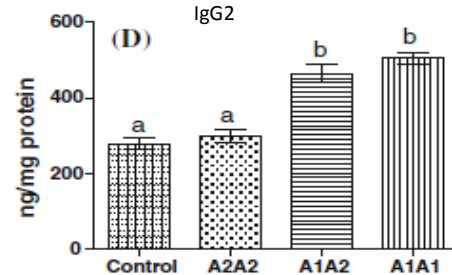
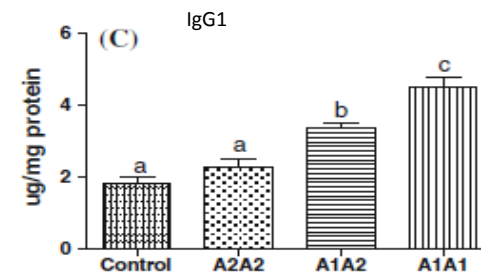
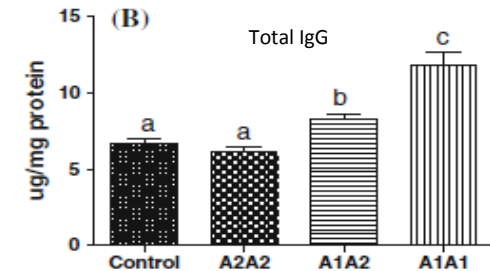
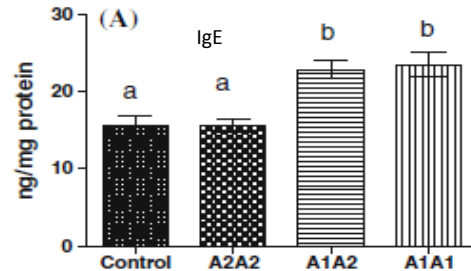
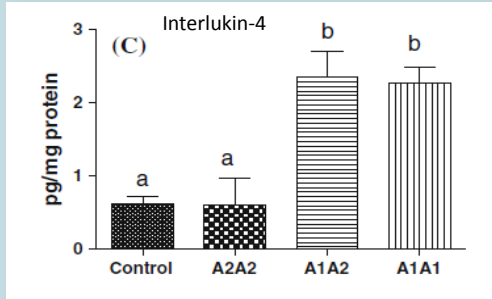
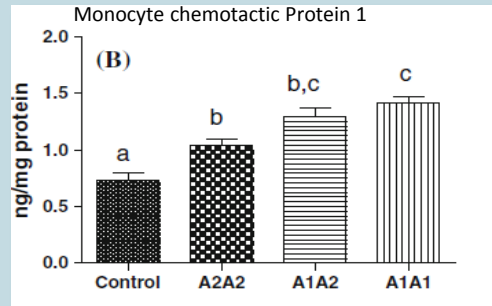


A1 vs A2 protein consumption resulted in:

- Increased GI inflammation (MPO)
- Increased transit time (GITT)
- Increased DPPIV – enzyme that breaks down BCM-7 but also controls stomach emptying and metabolic hormones.

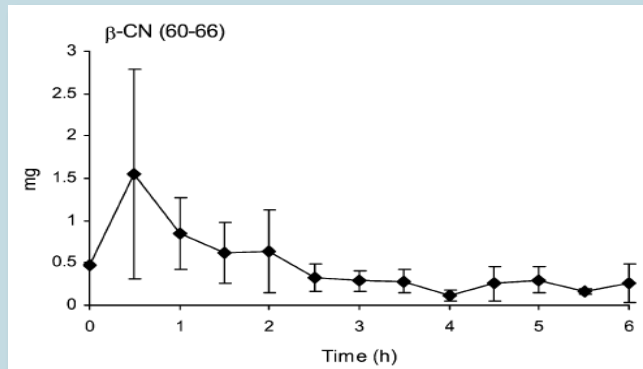
Ul Haq *et al* (2013)

- Comparative evaluation of cow β -casein variants (A1/A2) consumption on Th2-mediated inflammatory response in mouse gut. Haq MR, et al (2013) Eur J Nutr.
- A1 protein induced inflammation is BCM-7 mediated through established immune/cell pathways.



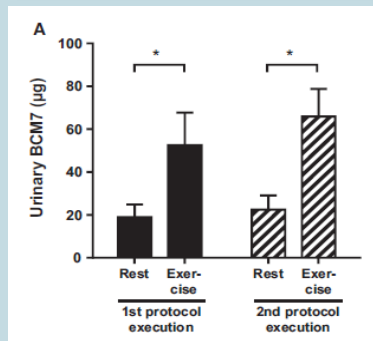
Boutrou *et al* (2013) and Ho *et al* (2015)

- Boutrou *et al* (2013) provide time line and amount of BCM-7 production following the consumption of milk protein.
 - Milligram amounts, sufficient to elicit biological response from tissue in the gut
 - Peaks at 30 min and present for four hours following intake of milk.



- Ho *et al* (2014) Preliminary Human Clinical Trial reports the potential link between A1 beta casein, GI inflammation and symptoms of intolerance

“a statistically significant positive association between abdominal pain and stool consistency was observed when participants consumed the A1 but not the A2 diet. Further studies of the role of A1 beta casein in milk intolerance are needed.” (Ho *et al*, 2014 and Pal *et al*, 2015)



JanssenDuijghuijsen *et al* (2016) [Physiol Rep.](#) 4 (20) reports detectable and increasing levels of BCM-7 in urine post exercise stress as a marker of intestinal permeability

Trivedi *et al* (2014) - Mechanism of BCM-7 interaction with human cells¹

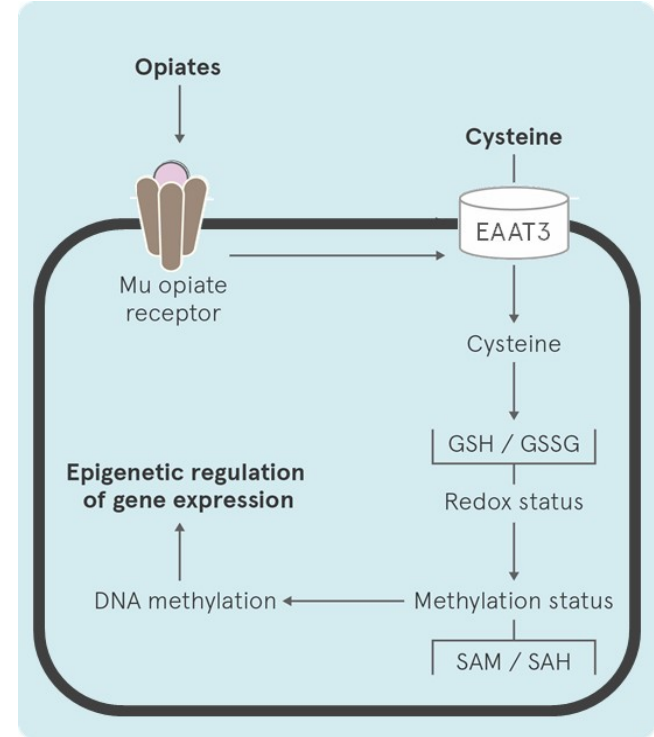
A mechanism of BCM-7's biological interactions with neuronal and gut epithelial cells was published in 2014

BCM-7 binds the Mu Opiate receptor which is coupled to the EAAT3 receptor

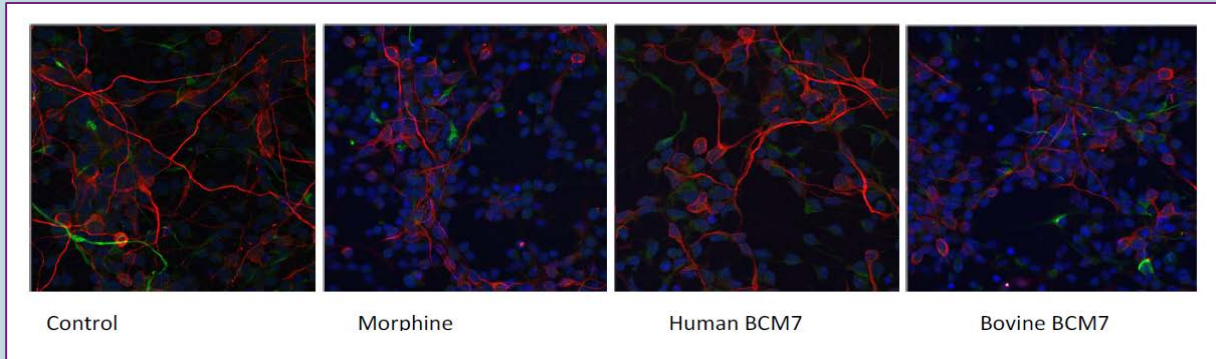
BCM-7 binding inhibits cysteine uptake by EAAT3 receptor, reducing the level of substrate for antioxidant production

Consequently cellular glutathione (GSH) levels decrease putting the cell under oxidative stress

Oxidative stress and subsequent inflammation triggers both changes in gene expression and epigenetic modification of DNA



Trivedi *et al* (2015)

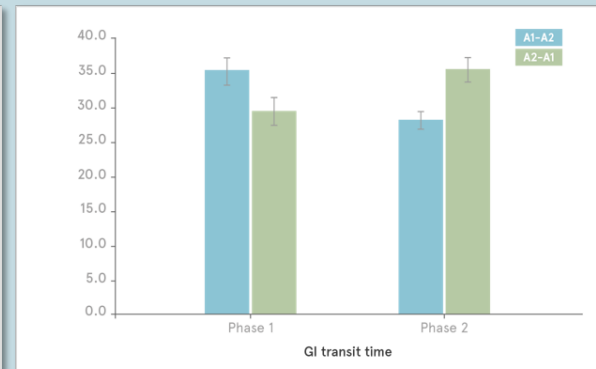
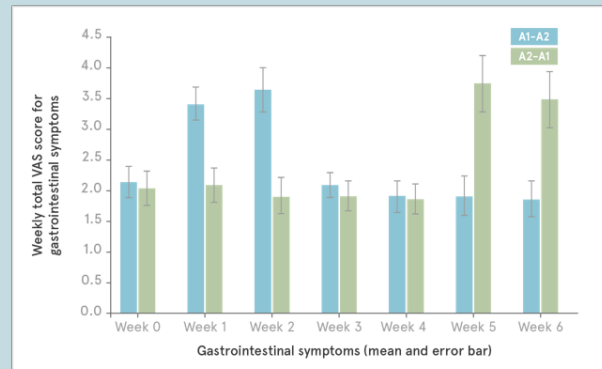
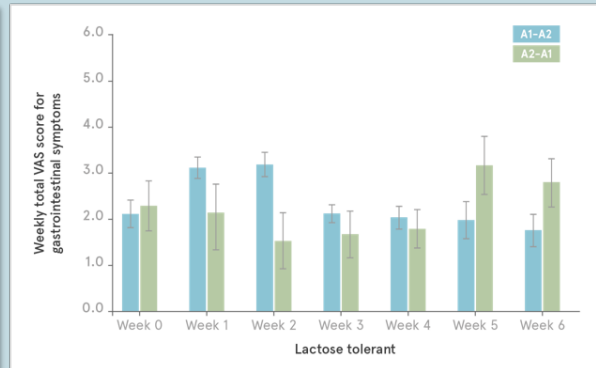
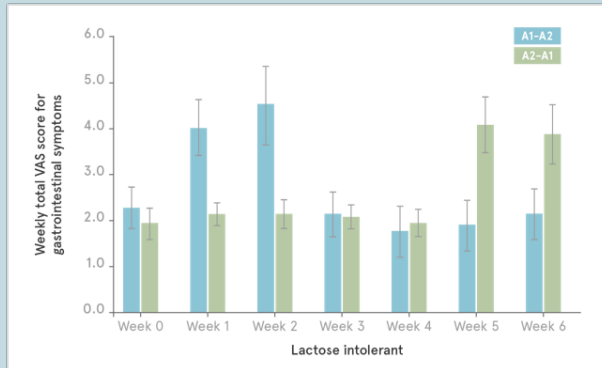


¹ Trivedi MS *et al*, Food-derived opioid peptides inhibit cysteine uptake with redox and epigenetic consequences. (2014) *J Nutr Biochem*. 25(10):1011-8.

1Trivedi M, Zhang Y, Lopez-Toledano M, Clarke A, Deth R. (2016) Differential neurogenic effects of casein-derived opioid peptides on neuronal stem cells: implications for redox-based epigenetic changes. *J Nutr Biochem*. Nov;37:39-46

- Neural stem cells were treated with BCM-7, BCM-9 (A2) and hBCM-7 (from human)
- BCM-9 (A2) was comparable to hHBCM-7 in its effects where as BCM-7 was closer in effect to positive control morphine.

Jianqin *et al* (2016) – Human Clinical n=45 Milk Intolerant



Plots of reported intolerance symptoms of participants consuming a2 Milk™ vs conventional milk in double blinded crossover clinical trial.

- (a) Participants confirmed as lactase deficient
- (b) Participants without lactase deficiency, and (Figure 3) is combined data

Gastrointestinal (GI) transit time also plotted for feeding groups and phases, showing increased (6hrs) transit time in conventional (A1/A2) fed groups compared to a2 Milk™ fed

Conventional milk reported to increase inflammatory and immune markers

Table 2 Results of serum and fecal laboratory tests

Variable	Sequence 1 ^a				Sequence 2 ^b				Mixed-effects ANOVA		
	Period 1		Period 2		Period 1		Period 2		Estimate ^c	SD	P-value ^d
	BL	PI	BL	PI	BL	PI	BL	PI			
Serum											
hs-CRP (mg/L)	1.00 ± 0.70	1.17 ± 0.64	0.97 ± 0.58	1.10 ± 0.58	1.03 ± 1.03	1.02 ± 1.11	1.01 ± 0.98	1.18 ± 1.04	0.0722 ^e	0.03746	0.0608
Hb (g/L)	141.7 ± 17.5	145.1 ± 17.0	136.7 ± 23.2	143.9 ± 16.4	142.8 ± 20.1	145.5 ± 17.7	137.5 ± 25.2	142.0 ± 18.1	−0.8654	1.6781	0.6088
IL-4 (ng/L)	11.8 ± 4.2	14.1 ± 5.2	11.1 ± 3.4	11.0 ± 3.2	11.9 ± 4.3	12.0 ± 3.7	11.8 ± 3.4	14.1 ± 4.6	2.5258	0.5338	<0.0001
IgG (g/L)	10.3 ± 2.1	11.6 ± 2.3	10.2 ± 1.7	10.6 ± 1.4	10.6 ± 2.1	11.1 ± 1.9	10.8 ± 1.8	12.2 ± 1.7	0.1426 ^e	0.03915	0.0007
IgE (IU/mL)	61.3 ± 29.0	69.8 ± 38.0	63.3 ± 30.1	66.2 ± 28.9	58.6 ± 31.2	60.7 ± 33.3	56.7 ± 31.3	64.4 ± 34.2	5.9688	2.5741	0.0253
IgG1 (µg/mL)	29.4 ± 31.3	37.4 ± 39.1	31.0 ± 33.1	30.3 ± 32.9	33.0 ± 28.3	28.5 ± 28.5	32.9 ± 27.2	37.4 ± 31.4	0.2424 ^f	0.07873	0.0037

- Reported in three animal studies in 2014
- Confirmed in clinical trials in participants reported and confirmed as milk protein or lactose intolerance
- Images taken with a pill endoscope (camera) demonstrate increased gut inflammation with A1 protein containing milk

a2 Milk™ associated with improved levels of SCFA; markers of healthy gut bacteria

Feces											
Acetic acid (%)	0.42 ± 0.15	0.42 ± 0.15	0.40 ± 0.14	0.46 ± 0.11	0.39 ± 0.19	0.46 ± 0.19	0.39 ± 0.17	0.36 ± 0.11	-0.0667	0.0226	0.0052
Propanoic acid (%)	0.18 ± 0.07	0.18 ± 0.07	0.17 ± 0.07	0.17 ± 0.07	0.17 ± 0.09	0.19 ± 0.13	0.18 ± 0.09	0.17 ± 0.07	-0.006 ^e	0.0187	0.7504
Butanoic acid (%)	0.17 ± 0.07	0.16 ± 0.07	0.16 ± 0.07	0.20 ± 0.08	0.17 ± 0.09	0.23 ± 0.09	0.17 ± 0.08	0.16 ± 0.05	-0.0515	0.0122	0.0001
Total SCFA (%)	0.76 ± 0.24	0.76 ± 0.24	0.72 ± 0.24	0.83 ± 0.19	0.73 ± 0.33	0.88 ± 0.33	0.74 ± 0.28	0.69 ± 0.18	-0.1289	0.03609	0.0009

^aSequence 1: A1/A2 → A2

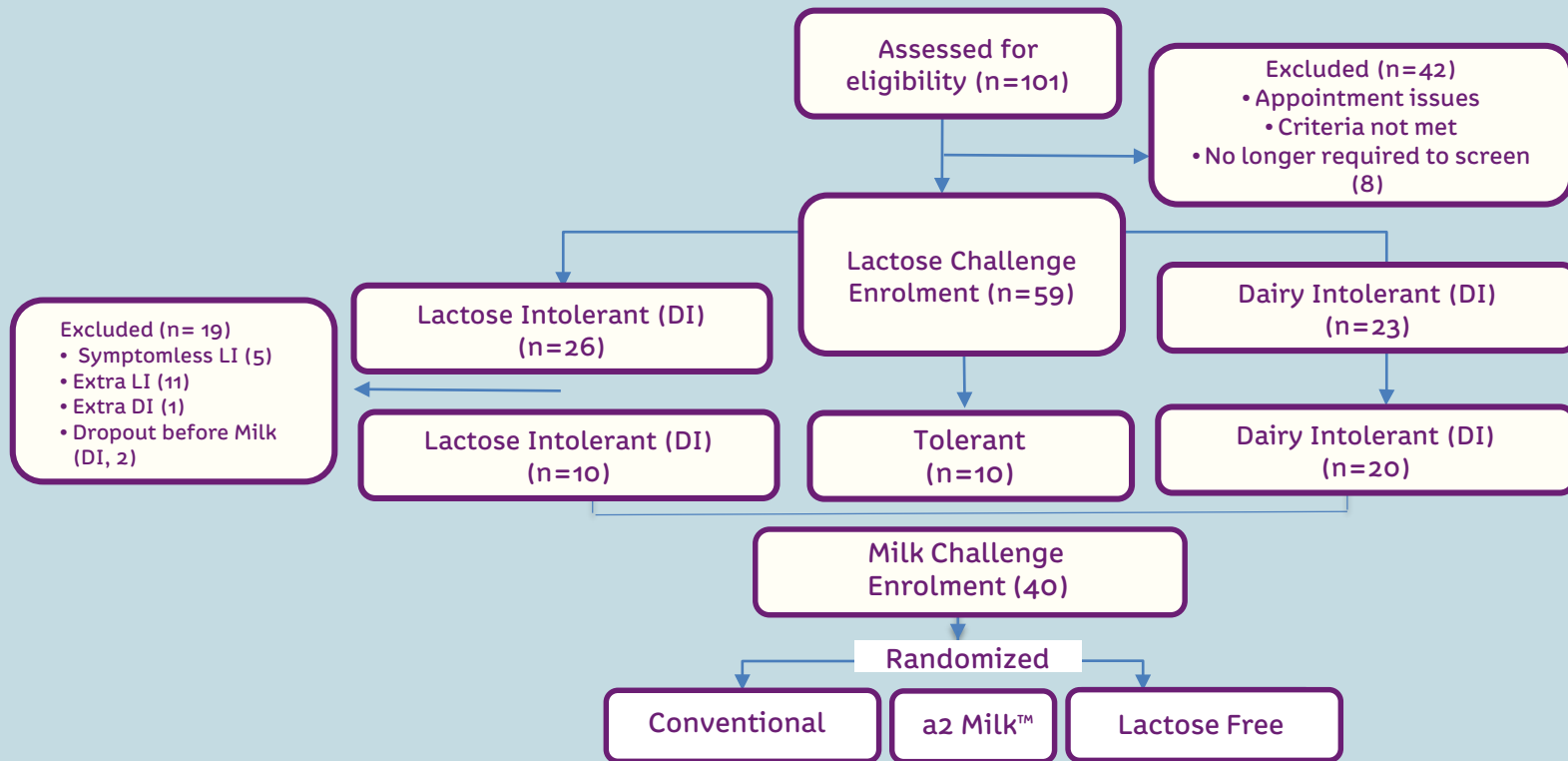
^bSequence 2: A2 → A1/A2

^cA1/A2 - A2

- Reported in three animal studies in 2014
- Confirmed in clinical trials in participants reported and confirmed as milk protein or lactose intolerance
- Images taken with a pill endoscope (camera) demonstrate increased gut inflammation with A1 protein containing milk

Clinical data reports a2 Milk™ vs. conventional milk associated with higher levels of 'healthy' gut bacteria.

HVN – aMiGo Trial (a2 Milk™ for gut comfort)

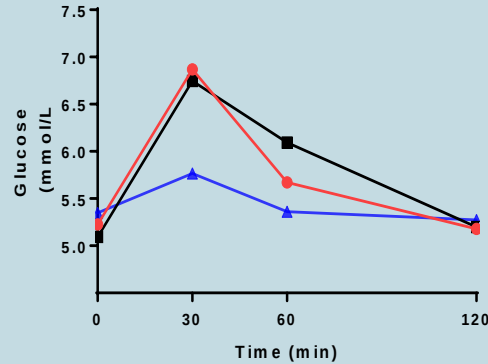
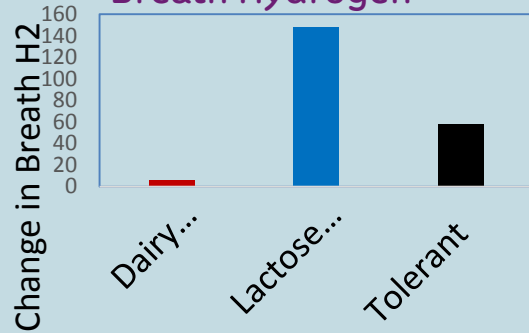


Participant Characteristics

Malabsorption

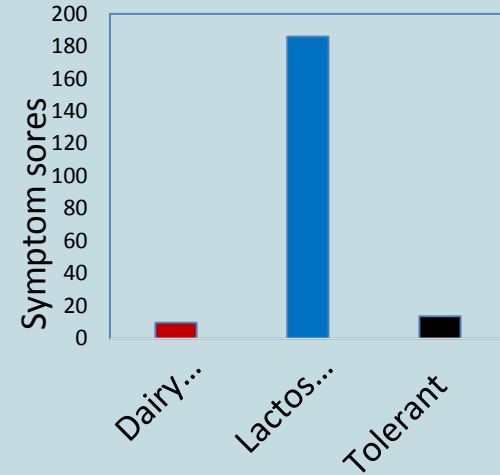


Breath Hydrogen



Symptoms (Flatulence, Bloating, etc.)

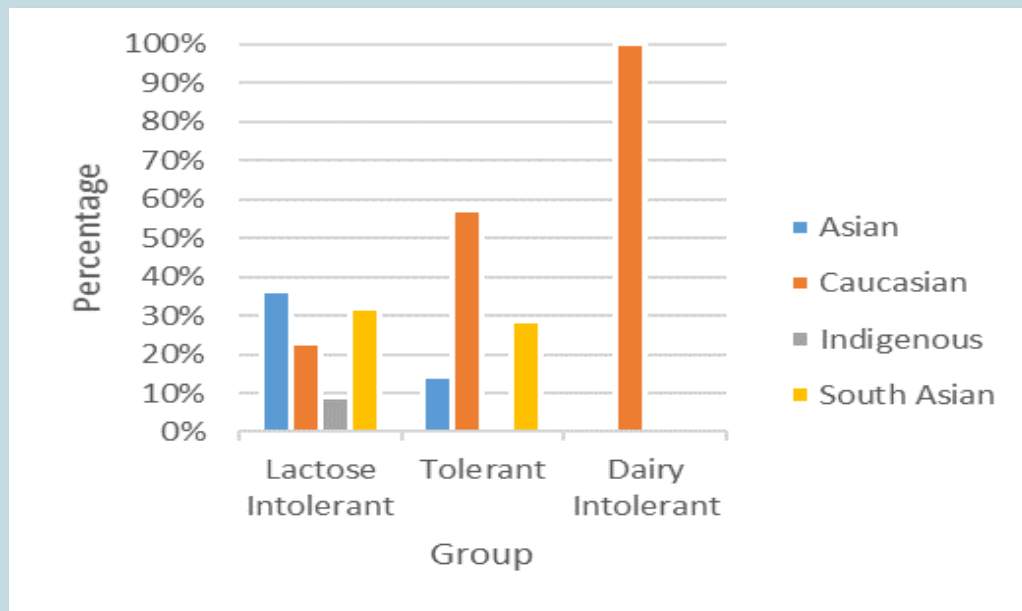
Symptoms at 3 hrs



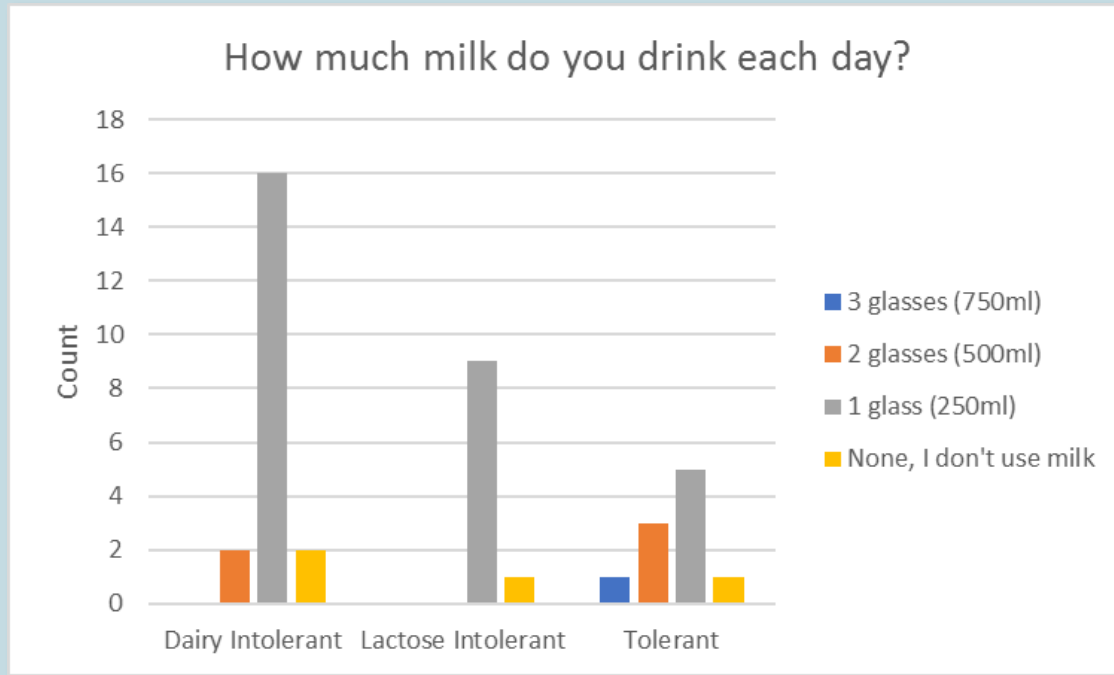
- Dairy Intolerant
- Dairy Tolerant
- ▲ Lactose Intolerant

Ethnic Distribution

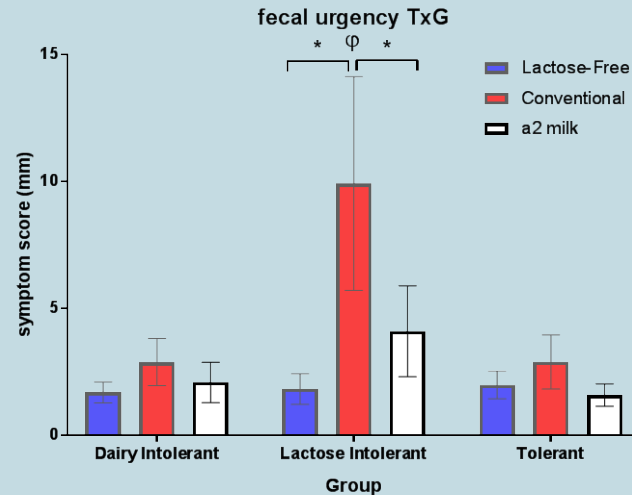
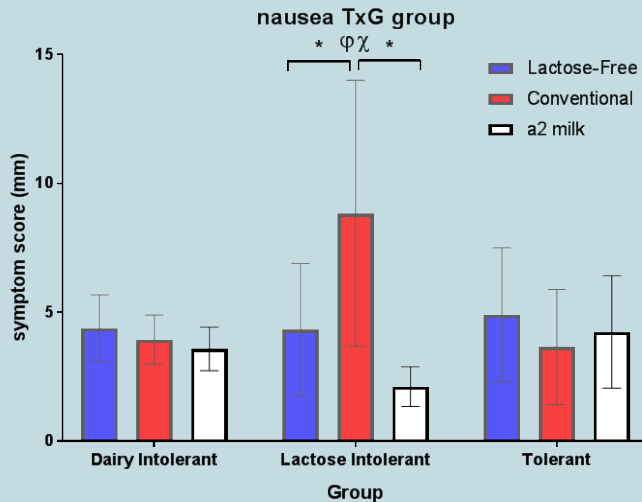
- No non-Caucasians in Dairy Intolerant group
- Fewer non-Caucasian absorbers



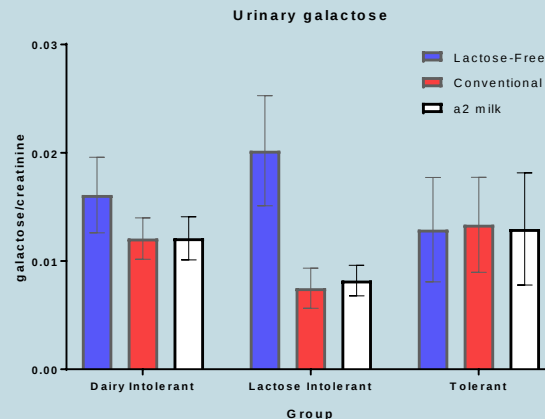
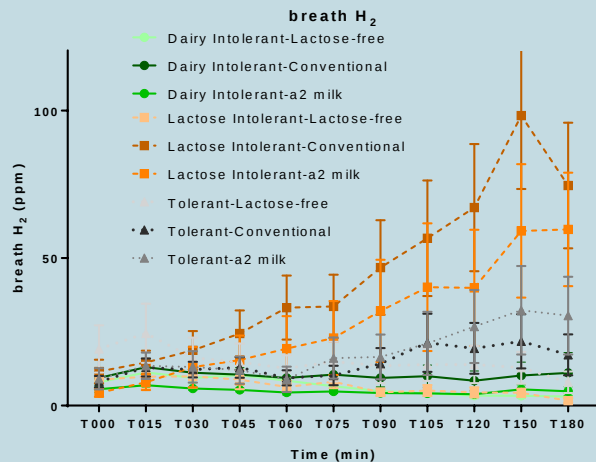
Dairy avoidance in dairy avoiders?



Lactose intolerant subjects experience greater symptoms with conventional milk



Malabsorption reduced with a2 Milk™



- Breath hydrogen decreased after a2 Milk™ compared to conventional
- Urinary galactose not different

Summary: The aMiGo Trial

- In lactose intolerant individuals, a2 Milk™
 - Reduced nausea and faecal urgency than conventional milk, similar to lactose-free
 - Breath hydrogen increased later and less after a2 Milk™ than conventional in lactose intolerant subjects

The Future

- Confirm and extend acute study observations around A1 protein free milk benefits to lactose intolerance.
- Examine and characterise the mechanism by which the effects are imparted
- Identify breathalomic or metabolomic markers to identify respondent individuals.
- Determine the proportion of respondents of selected population groups or market
- Study medium to long term influence on metabolic and inflammatory markers and outcomes

Acknowledgements

The High-Value Nutrition Science Challenge

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Ms Suzanne Simpson (a2MC)

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