

A1 and A2 beta-casein: Searching for common ground and moving the science forward

Summary Report for Health Professionals

Since the 1990s much evidence has arisen of possible adverse effects on human health of the A1 beta-casein protein found in cows' milk. To many key health stakeholders and nutrition professionals, however, the science behind the differences between A1 and A2 variants remains unclear and this has led to widespread confusion about the importance and relevance of existing and emerging research.

Major reviews of existing scientific literature on A1 and A2 beta-casein variants were published in 2004 (NZFSA report) and 2009 (EFSA report), but since then more data has been published and clinical trials conducted. On 16 June 2016 the Nutrition Society of Australia hosted a closed expert meeting at which researchers in the field presented and summarised the latest findings to key opinion leaders in health and nutrition, gastroenterology, NGOs, public health and the dairy industry. Funding for the workshop was provided by The a2 Milk Company (Australia) Pty Ltd.

SESSION 1: INTRODUCTION

An introduction to beta-casein science

Professor Keith Woodford, PhD, was formerly Professor of Agribusiness at Lincoln University, New Zealand, and is now an independent consultant working internationally with agri-food companies and government bodies. His 2007 book 'Devil in the Milk' focused public attention on health concerns about A1 milk protein.



Professor Woodford provided an introduction to beta-casein science, explaining that a 250mL glass of milk typically contains 30-38g of solids, of which 7-10g is protein, most of which in turn is casein. Within the casein are three key types of protein -- alpha, beta and kappa -- with beta-casein constituting about 2-3g (or half a teaspoon). There are two main types of beta-casein, A1 and A2, with A2 known to be the original form and A1 known to be a mutation that appeared possibly 5000 years ago in cattle of European origin. Most supermarket milk contains a mix of A1 and A2 proteins. Milk free of A1 beta-casein is found in goats, sheep, purebred Asian and African cattle, and also DNA-tested "A2" cattle selected to produce milk for The a2 Milk Company™. Human milk is also free of A1 beta-casein.

Though the defining distinction between A1 and A2 beta-casein is the substitution of just one of the 209 amino acids that make up the beta-casein proteins, Prof Woodford said that single amino acid had profound effects in the way the protein is digested. During normal human enzymatic digestion of the A1 protein, a peptide called beta-casomorphin-7 (BCM-7) is released. But no such release occurs during digestion of the A2 protein.

BCM-7 has certain opioid characteristics and attaches to mu-opioid receptors. Researchers have found that BCM-7, like many mu-opioids such as codeine, can inhibit peristalsis and slow the transit of food in humans and animals.^{1,2} BCM-7 is known to cross into the circulatory system of babies and children fed milk formula containing A1 beta-casein,^{3,4} and some babies with high levels of BCM-7 have displayed delayed psychomotor development.⁵ BCM-7 has been found in the urine of babies fed formula and is also known to cross the blood-brain barrier.⁶ Animal studies have linked BCM-7 to gut inflammation⁷ and immune response.⁸ Considerably higher levels of the enzyme DPP4, which breaks down BCM-7, have also been found in the jejunum of rats fed A1 milk, compared with those fed A2 milk.¹ Prof Woodford said: "Gliptins are a major and relatively new group of drugs used in the management of Type 2 Diabetes Mellitus. Whereas they inhibit DPP4 activity, here we found that A1 milk increases DPP4 enzyme levels. The clinical significance of those findings awaits further investigation."

There is evidence that BCM-7 oxidises LDL⁹ and that A1 beta-casein is causal in relation to arterial plaque in rabbits.¹⁰ He also referenced "powerful population epidemiology" that links A1 beta-casein with heart disease.¹¹

Epidemiological evidence including the implications and interpretation of the ecological evidence with key conclusions

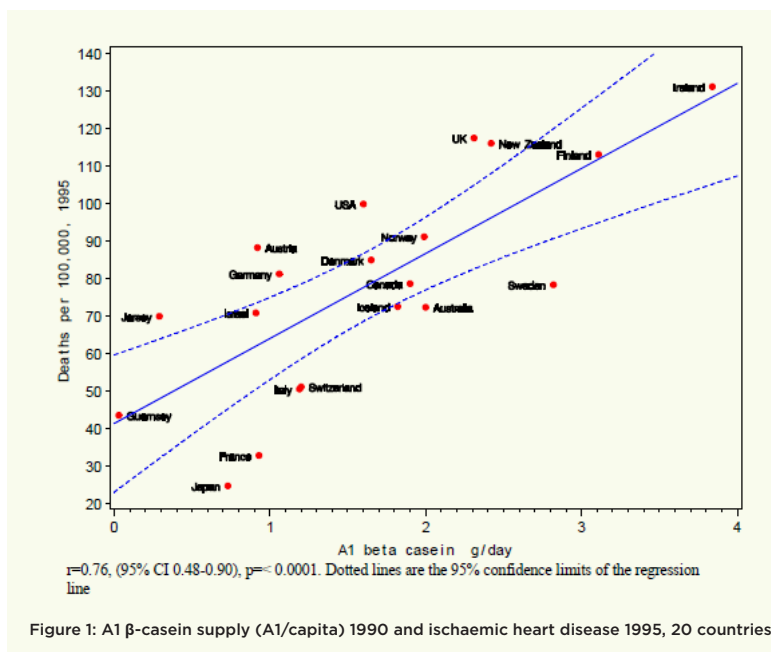
Professor Boyd Swinburn is Professor of Population Nutrition and Global Health at the University of Auckland and Alfred Deakin Professor and Co-director of the World Health Organisation Collaborating Centre for Obesity Prevention at Deakin University in Melbourne. His primary field of work is obesity prevention and food policy. In 2003, he was commissioned by the New Zealand Food Safety Authority to conduct a review of literature on health issues surrounding A1 and A2 beta-casein and to make recommendations for future action by government agencies.



Professor Swinburn's presentation focused on epidemiology and public health. He said the ecological relationships between A1 beta-casein and certain diseases were "the most compelling and the most misunderstood and misinterpreted part of the epidemiology".

His address highlighted the findings and implications of the seminal 2003 Laugesen and Elliott ecological study,¹² which examined food consumption data from 20 "health care affluent" countries to investigate any correlations between the rate of Type 1 Diabetes Mellitus (T1DM) and Ischaemic Heart Disease (IHD) mortality.

"The ecological relationships are the most compelling and the most misunderstood and misinterpreted part of the epidemiology."



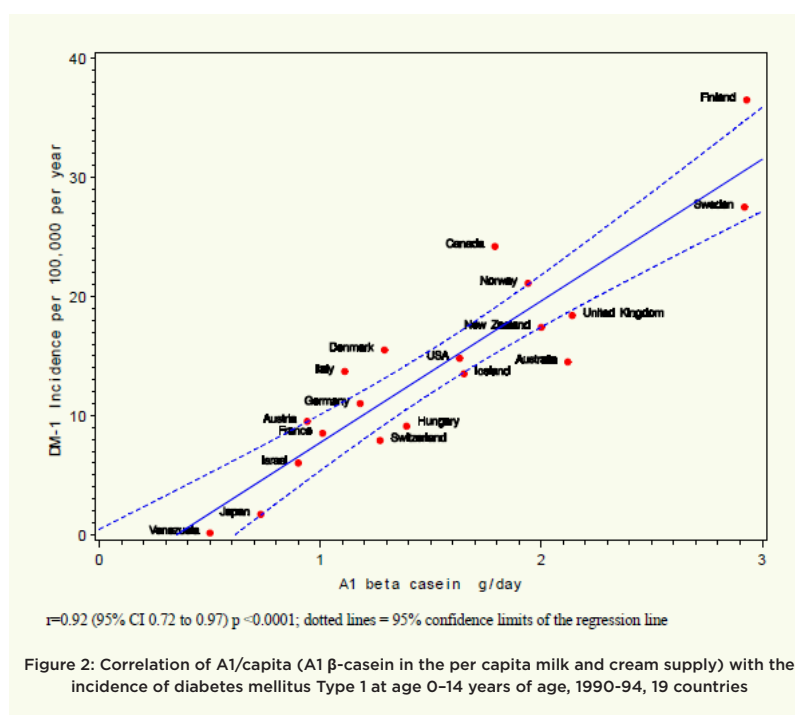
The pair examined 18 independent variables placed on the horizontal axis of the IHD chart but found the strongest relationship – with an r value of 0.76 – was the measure of grams per day of A1 beta-casein from milk and cream. Countries with the highest A1 consumption also had the highest rates of IHD deaths. Prof Swinburn said $r=0.76$ was a strong correlation for a condition that was highly multifactorial.

Over a 20-year period the correlation was consistently stronger than that with smoking and consumption of alcohol and saturated fat – all known risk factors for IHD. He said one of the strengths of those ecological studies was the five-fold range between countries in both A1 beta-casein consumption per capita and incidence of IHD mortality.

A similar search for any correlation between A1 consumption and Type 1 diabetes incidence – in which there was a fivefold range of A1 consumption and 280-fold range in T1DM incidence – produced an "extremely high" r value of 0.92, the strongest of 15 correlates. Prof Swinburn said although it was possible the correlation was created by a third – as yet unidentified – common factor, none had been proposed that could create an r value of 0.92. He concluded: "We have to go on the balance of probabilities that it may well be causal."

He said scientists and science funders should be pursuing the hypothesis raised by Laugesen and Elliott's work far more vigorously than they were. He listed a number of reasons why this was not taking place: scientists were often conflicted by commercial interests and reluctant to discard "pet theories"; public good agencies were reluctant to fund research that might lead to commercial outcomes; the mainstream dairy industry was wary of the risk it carried to their business; and governments were both highly susceptible to commercial interest lobbies and also very conservative in the presence of evidence uncertainty.

"Ecological studies get tossed out by commentators, I think quite unfairly because they do tell us good information."



SESSION 2: CURRENT RESEARCH - GUT HEALTH

Comparison of the effects of milk beta-casein types on gut physiology and behavioural health

Professor Peter Gibson is the Director of Gastroenterology at Alfred Health and Monash University. He has an interest in diet and how it can be used to influence disease pathogenesis and symptoms.



Professor Gibson said the A1/A2 debate was very important for the clinical practice of gastroenterologists and GPs. Many of his patients with functional gut disorders and food intolerances had reported their condition improved when they switched to a2 Milk™, but he said: “We really need to know whether there is anything in the story.”

He said discussion of food-related exorphins, peptides released with opioid actions in the gut, was often controversial, with research findings on those produced by gluten particularly igniting great passion. Compared with gluten, which had many confounding factors, the A1/A2 milk issue was a very “clean” model because of the single difference being the release of the BCM-7 peptide, and therefore very useful to examine whether exorphins exerted an effect on psychological status and gastrointestinal (GI) physiology.

Prof Gibson said the real issue was whether the BCM-7 peptide released during A1 milk digestion could actually produce effects in the gut and elsewhere. He reviewed three recent studies on the effects of A2 versus A1/A2 milk on humans. An exploratory study suggested milk was a factor in childhood constipation but was unable to show A1 and A2 beta-caseins were causally involved,¹³ while a crossover study at Curtin University showed some hint of differences between A1 and a2 Milk™ in stool consistency.¹⁴ He criticised the methodology and conclusions of a 2016 Chinese study that found significant differences in gastrointestinal outcomes in people with self-reported intolerance to traditional cows' milk containing A1 beta-casein compared to drinking a2 Milk™.^{15,16}

Prof Gibson explained how his Monash team had sought funding from The a2 Milk Company™ for an experimental study now under way that would provide high quality evidence on whether there were differences after exposure to A1 vs A2 beta-casein on intestinal physiology, GI symptoms in individuals with IBS, and behavioural characteristics such as cognition, depression, anxiety and sleep patterns. To provide the greatest contrast between the milk proteins the study is comparing a2 Milk™ with pure “A1A1” beta-casein milk. The project is using Smart Pills to gain definitive measures of GI transit times and he expects results in 12 months.

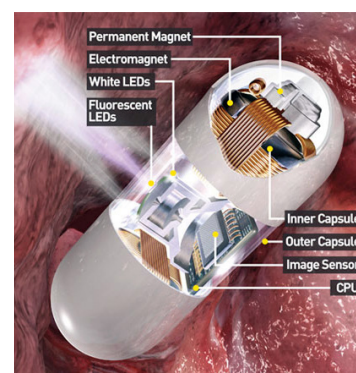


Figure 3: The Smart Pill

Comparative effects of A1 versus A2 beta-casein on gastrointestinal measures: a blinded randomised cross-over pilot study

Associate Professor Sebely Pal from Curtin University's School of Public Health in Perth, has spent more than 20 years studying nutrition and chronic disease in Australia, specialising in obesity, diabetes and cardiovascular disease.



Professor Pal said milk consumption had been decreasing globally in recent years and in many countries failed to meet dietary intake guidelines. Reasons for this include an increase in allergies as well as real or perceived lactose or milk intolerance, although studies have suggested the prevalence of lactose intolerance may be much lower than previously thought. GI intolerance responses may instead be caused by other natural components of milk, and beta-casein in milk has been identified as a key factor.

She said persuasive evidence had emerged of possible differences on GI health when comparing A1 and A2 beta-casein consumption. Research with rats or mice found GI transit was slowed among those fed A1 beta-casein; production of the enzyme dipeptidyl peptidase-4 (DPP4) was increased by A1 beta-casein in the GI tract, which in turn could be responsible for decreased gut motility and slowed gastric emptying. GI production of the inflammation marker myeloperoxidase (MPO) was significantly increased; cytokine, immune and inflammatory activity in the colon was increased; and mucous production was increased. Human intestinal in-vitro experiments showed BCM-7 altered intestinal lymphocyte proliferation and induced rapid secretion of intestinal mucus.

She said the 2014 Curtin University exploratory pilot study that she co-authored¹⁴ was the first in vivo research comparing the effects of dietary A1 versus A2 beta-casein in milk on GI symptoms in human adults. The Curtin project was a double blinded eight-week crossover trial in which 41 participants who drank 750mL of either A1 or A2 beta-containing milk daily were assessed for disease markers in blood, GI symptoms, stool consistency and gut inflammation. Slightly higher measures of GI symptoms of bloating, abdominal pain, flatus and difficulty in voiding were reported on the A1 beta-casein in milk diet, but among the milk-intolerant subset, bloating, abdominal pain and voiding difficulty were all markedly higher.

Correlation between faecal calprotectin, a marker for GI inflammation, and measures of intolerance found more inflammation associated with bloating, abdominal pain and flatus when using A1 milk compared with A2. She said some faecal calprotectin values were high among milk-intolerant users on the A1 diet. User-reported measures of stool consistency on the Bristol Stool Scale (BSS) showed higher BSS values when users drank A1. A strong correlation was found between abdominal pain and softer stools on the A1 diet, but the relationship did not exist on A2.

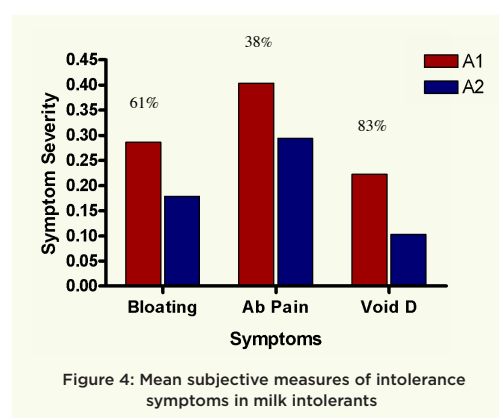


Figure 4: Mean subjective measures of intolerance symptoms in milk intolerants

What is the evidence for milk increasing mucus production?

Dr Sue McGlashan is a senior lecturer in the University of Auckland's Department of Anatomy and Medical Imaging and is a member of the medical committee of the Auckland Medical Research Foundation.

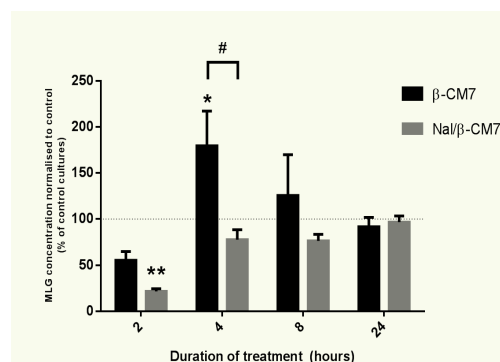


Dr McGlashan detailed the findings of a new in vitro study examining the evidence that milk consumption increases respiratory mucus production. She said anecdotal evidence had existed for many years that drinking milk made users "phlegmy" and that one of the hypotheses relating to milk was that peptides released during digestion of caseins might regulate mucin expression and activity in the gut. She discussed the findings of earlier gut mucus studies from 2002 to 2015 showing milk hydrosalates or BCM-7 and other opioid peptides increased mucin production in rat jejunum tissue with the action blocked by naloxone, suggesting action through mu-opioid receptors.^{17,18} Human and rat goblet cell line in vitro tests also showed an increase in mucin gene expression and secretion. This too was inhibited by naloxone.¹⁹

In 2009 Dr McGlashan produced a "hypothesis" paper with ENT surgeon Jim Bartley, who had previously observed that when a subset of patients suffering chronic rhinosinusitis were placed on a dairy-free or wheat-free diet their symptoms improved and mucus production decreased. The pair therefore conducted in vitro studies to look at whether the mechanism reported in the gut epithelium could be examined in the respiratory epithelium.

They replicated Zoghbi's work, then switched to respiratory research using human bronchial epithelial cells. Four hours' exposure to BCM-7 triggered a robust increase in mucus production by goblet cells, and again that effect was inhibited with naloxone.

Dr McGlashan said the question remained of why a subset of Bartley's patients responded to a dairy-free diet and others did not and she suggested it might be related to gut permeability. She said many questions remained unanswered, but the data supported the belief that BCM-7 might be implicated in mucus production.



SESSION 3: CURRENT RESEARCH - INFANT HEALTH & DIABETES

Implications of beta-casein variants in Infancy

Dr Ashwantha Kumar Enjapoori gained his PhD in lactation biology and is a post doctorate fellow at Deakin University. His research has focused on milk proteins as well as BCM peptides in human breast milk.



Dr Enjapoori's interest is in the proteins that make up part of the macro- and micronutrients of human milk, and in particular the beta-casein, which is A2-like. He said caseins serve as the prime nutritional source of calcium, phosphate and amino acids to meet infant's growth and energy requirements and also transport calcium phosphate through the mammary milk system. He said a range of human BCM peptides were cleaved from human beta-casein through enzymatic action and had been detected in raw and pasteurized breast milk, breast milk-fed infant's plasma, urine and jejunal fluids, and human brain stems. Their functions are known to include immuno-modulation and as opioids and antioxidants. The release of human BCM-7 also sends a satiety signal to the brain, halting food intake.²⁰

Dr Enjapoori's work has been aimed at identifying which BCM peptides are released *in vivo* in human lactation. A wide range of breast milk samples were collected, from preterm to day 459, and a mass spectrometry-based peptidomic technique was used to detect peptides, revealing they were released in a specific sequence over time rather than randomly. The conclusion was that the release of BCM peptides before infant's intake may provide selective advantages for both infant and mother, so future research will aim to identify their specific functions.

Chaperone activity of A1 vs A2 beta-casein

Professor John Carver is Director of the Research School of Chemistry at the Australian National University in Canberra. His research interests include protein structure and molecular chaperones.



Prof. Carver explained that casein proteins associate together to form casein micelles, which are large enough to scatter light and result in the opaque white colour of milk. Two of the four caseins (kappa and alpha-s2 caseins) form cytotoxic amyloid fibrils, the same type of species that are also found in the proteinaceous plaque deposits associated with diseases such as Alzheimer's and Parkinson's and in systematic amyloidosis diseases, including in cattle. However, casein micelles are very good at stabilising kappa- and alpha-s2 caseins from forming amyloid fibrils and therefore causing toxicity. The ability of alpha-s1 casein and beta-casein to prevent other proteins from aggregation, including amyloid fibril formation, is described as a chaperone effect; beta-casein is a very good molecular chaperone^{21,22}.

Prof. Carver's research has found that A2 beta-casein forms smaller micelles and is also a more efficient molecular chaperone than A1 beta-casein. Thus, there is more "free" A2 beta-casein present in solution to act as a molecular chaperone²³. This may allow A2 beta-casein to prevent more efficiently the coagulation of milk (i.e. precipitation of casein proteins) upon addition of rennet during cheese production, which may explain the relative rarity of A2 hard cheese and could provide the historical reason for the prevalence of A1 milk. He suggested: "Farmers, generations ago, may have chosen cows that produced a form of milk that made cheese more easily and possibly produced more milk. Could this also affect the ability of A1 milk to be digested because it forms a stronger micelle? Maybe this also causes some of the health effects of A1 milk relative to A2, i.e. A1 beta-casein is more difficult to digest."

Beta-casein variants & Type 1 Diabetes

Professor Karen Dwyer is the Professor and deputy head of the school of medicine at Deakin University. Her clinical research interests projects focus on diabetes.

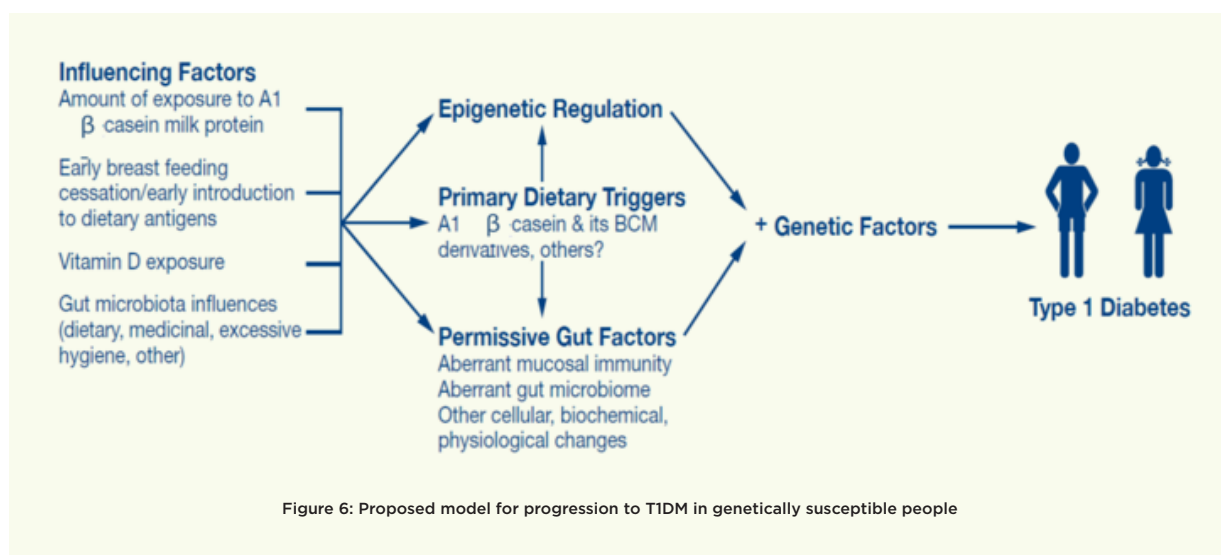


Prof Dwyer said the incidence rates for T1DM were increasing worldwide with high rates in New Zealand and Australia, for example. Although genetic causes are well known, there is increasing interest in the role of environment, with an intriguing rise in T1DM incidence as migrating populations move from low-risk to high-risk areas. Such environmental factors could include viruses, the timing of introduction of solid food for infants and the role of cows' milk, particularly A1 beta-casein.

Some animal studies have been conducted to try to support epidemiological evidence, but have been thwarted by issues of methodology. As a result, Deakin researchers conducted their own studies using NOD (specially bred non-obese diabetic) mice fed diets supplemented with either A1 or A2 beta-casein, hypothesising that the A1 diet would augment the incidence of T1DM. The researchers were particularly interested in observing the incidence of T1DM in NOD mice over multiple generations.

Three weeks after birth the first generation of mice were put on separate diets with either A1 or A2, then at sexual maturation they were bred to produce new generations that remained on the selected diet. In the first three generations, F0-F2, no significant difference in T1DM was noted. But a significant divergence developed in the F3 generation, with the incidence of diabetes doubling at age 20 weeks in A1 beta-casein fed mice. The researchers then examined the F4 generation at 10 weeks of age prior to clinically evident diabetes. The female mice fed the A1 diet were significantly lighter than those fed A2 and their fasting blood glucose levels were elevated. The researchers then examined the islets of the 10-week-old female mice and found most of those fed A1 had insulinitis, the majority graded moderate to severe whereas in the A2-fed mice the majority had no evidence of insulinitis. Rates were lower among males.

This work raises questions of whether BCM-7 is involved in T1DM onset, whether it has any direct toxicity on islet cells and whether it is affecting gut mucosal immunity and alteration in the gut microbiome. The Deakin researchers are now searching for this evidence focusing on epigenetics and their early findings suggest there is global hypomethylation of the pancreas, associated with a reduction in the insulin receptor gene expression.



SESSION 4: CLINICAL IMPLICATIONS AND THE GAPS IN RESEARCH

Gastrointestinal food intolerance: Clinical correlates, challenge findings and implications for the role of A1/A2 beta-casein variants

Dr Robert Loblay is the director of the Allergy Unit of the Department of Clinical Immunology at the Royal Prince Alfred Hospital. His clinical and research interests include food allergy and food intolerance.



Dr Loblay discussed non-immunological food intolerances that are triggered by naturally-occurring non-nutrient chemical substances in food. He said about 15 years ago clinics received a "flood" of children with autism and the idea was popularised that autistic children were sensitive to gluten and casein. As a result, many children were put on gluten-free, casein-free diets, but a meta-analysis in 2008 showed there was poor evidence for the efficacy of those complementary and alternative therapies.²⁴

Dr Loblay said in 2004 and 2005 NSW students examined possible links between "leaky gut" and autism spectrum disorder and also whether urinary peptides could explain the pathophysiology of ASD. He said they found no significant difference between ASD and non-ASD children in the level of gluten exorphins and BCM-7 they excreted.

ROUND TABLE:

Prof Andrew Sinclair, Prof Gibson, Prof Swinburn, Prof Carver, Prof Dwyer, Prof Woodford, Associate Prof Pal, Dr Loblay and Dr Enjapoori took part in a round-table discussion on issues surrounding A1 and A2 beta-casein.

A Queensland paediatrician spoke from the floor to relate his experience with more than 100 patients who had been moved to a2 Milk™ in the past 12 months. “Almost all” had benefited from the switch, including those suffering milk allergies and reflux, children with post-antibiotic gut, Asperger’s, and constipation and diarrhoea. He said: “Whatever the hell the difference is, (a2 Milk™) makes a dramatic improvement in average kids with average problems.”

But Prof Gibson said such anecdotal evidence was of limited value in changing medical practice, which was inherently conservative: “What we want is a scientific basis for it in randomised controlled trials so we can influence all doctors.” Dr Loblay expressed scepticism over claims of success for “fad” dietary restrictions, saying deeper investigation usually revealed patients had changed many other components of their diet at the same time, with those other changes generally providing the real benefits.

An audience member said she was worried that adverse research findings on pure A1 beta-casein were wrongly viewed by the public as applying to commercially available milk, which was an A1/A2 mix. Prof Gibson said his experiments were designed only to prove an effect and a concept, so they required the use of 100% A1 milk. He said researchers had no control over the way the media disseminated information, and also expressed irritation that the funding of research by companies such as The a2 Milk Company™ created a perception of possible bias. In fact no other companies would fund such research, he said.

An independent consultant, Simon Brooke-Taylor, said a “black hole” existed in knowledge of how endogenous opioids react in the gut and that understanding this could unlock an understanding of how the exogenous opioid peptides like the BCMs worked. Prof Sinclair said a similar gap in knowledge also existed on the issue of whether BCM-7 was normally present in the urine and blood of children.

Prof Woodford said the issue of peptide excretion was important because the influential 2009 EFSA review had firstly declared it was highly unlikely that any BCM-7 could get into the circulatory system, then developed many of its arguments on that basis. In fact the work of Sokolov⁵ proved BCM-7 did find its way into urine and could do so only through blood. He was critical of the lack of knowledge displayed by some commentators. “If one is going to be out there communicating, there is a responsibility to become aware of the literature.”

Prof Swinburn used the experience of Sudden Infant Death Syndrome (SIDS) treatment to illustrate the tension between epidemiology and the demand for knowledge of scientific mechanisms. He said when epidemiology 20 years ago linked SIDS with sleeping positions and second-hand smoke, scientists balked at suggestions that parents be urged to place babies on their back to sleep and reduce infants’ exposure to cigarette smoke, insisting that the mechanisms of the possible risks should first be understood. Yet when those recommendations were issued anyway, SIDS rates had fallen despite a lack of knowledge on mechanisms. He said a parallel situation existed with A1 milk: changing herds had no downside and money was better spent on an advocacy campaign for dairy farmers to change herds than on scientific research to understand the mechanisms of A1 beta-casein’s effects.

Associate Prof Pal said that given most Australians were failing to meet the dietary requirements for milk, and that digestive discomfort was the main reason most people gave up milk, a switch to A2 was a good way to boost individuals’ milk intake.

But Prof Gibson cautioned that while the epidemiological data was “stimulating”, it should not be used as a basis to try to move the whole community to A2 milk. He said that without adequate data, the National Heart Foundation had a generation ago warned people to eat polyunsaturated margarine and avoid eggs, both of which produced poor outcomes.

RESEARCH GAPS

What are the greatest research needs now on the A1/A2 issue? Panel members gave a variety of answers.

- **Dr Enjapoori:** Gain more information on the mechanisms of BCM-7 transfer to biological systems.
- **Prof Dwyer:** Explore gut microbiota composition, given that so much literature links it with diabetes, heart disease and neurological disease.
- **Prof Woodford:** Determine the effect of A1 beta-casein on gut transit times in humans to test the “very strong” hypothesis that the passage of food is slowed down by BCM-7.

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