Beta casein A1 and A2 in milk and human health

Report to New Zealand Food Safety Authority

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EXECUTIVE SUMMARY

Background

There are several genetically-determined variants of β-casein, the protein which constitutes about 25-30% of cows' milk proteins. One variant, A1 β-casein, has been implicated as a potential etiological factor in type 1 diabetes mellitus (DM-1), ischaemic heart disease (IHD), schizophrenia, and autism. Another variant (A2 β-casein) has not been implicated in these diseases.

It is known that nutrition in early life has important health consequences in both childhood and adulthood. Cows' milk is a basic food for most infants and children and a common food for adults in most western societies. Therefore, if some components of milk are causative or protective of the diseases mentioned, it would have major public health implications.

The evidence to support the hypothesis that the A1/A2 composition of milk is an etiological factor in these diseases is reviewed.

Type 1 diabetes mellitus

There is a consensus that type 1 diabetes mellitus (DM-1) is caused by one or more environmental triggers which, in genetically susceptible people, promotes an autoimmune process that destroys the insulin-secreting, pancreatic β-cells. The evidence that A1 β-casein is one such trigger comes mainly from ecological studies. The strength of the correlations between countries of their A1 β-casein consumption and their incidence rate of DM-1 is extremely high, although such correlations cannot establish cause and effect and are subject to bias. The clinical studies available (mainly case-control studies of markers of immune reaction) are not very helpful in establishing cause and effect relationships because people with DM-1 (and other autoimmune diseases) have increased immune reactivity to many different antigens. The results from studies using animal models of DM-1 are mixed. The best-designed of the animal studies showed very little effect of a diet high in A1 β-casein on the development of diabetes. It is known that A1 β-casein

is cleaved enzymatically in the gut to produce a molecule (β casomorphin-7) which has some morphine-like actions in the body and it is postulated that this may influence the immune surveillance. A2 β-casein, the other main casein variant, does not undergo this cleavage and is not implicated in the disease processes.

Cardiovascular diseases

Ischaemic heart disease (IHD) and stroke represent the clinical outcomes of pathological processes that occur over decades (atherosclerosis) and acutely (thrombosis, arrhythmias). These processes are multi-factorial and several risk factors have been well established (smoking, high blood pressure, high cholesterol etc).   The evidence that a high intake of A1 β-casein is also a risk factor for IHD rests mainly on the same type of ecological data that the DM-1 case rests on. The correlations, while not as high as for DM-1, are still impressive for such a multi-factorial disease.   The available clinical evidence is sparse and unhelpful in determining whether this is a true cause and effect relationship. One animal study showed some support for the atherogenic nature of a diet with a very high A1 β-casein supplementation, but the results were far from conclusive and there is difficulty on translating animal studies to human health.

Neurological disorders

There have been several poorly-controlled clinical trials of casein-free, gluten-free diets in people with autism. In general, the diets seem to reduce some of the autistic behaviours, but the bias inherent in the studies (especially lack of blinded assessment) may explain some of the findings. The evidence that A1 β-casein is related to schizophrenia is very scant.

Implications

All the conditions discussed are major contributors to mortality and morbidity, so any dietary factor that could reduce the burden they impose should be taken seriously and examined for potential public health and clinical recommendations. It is abundantly clear that much more research is needed in all of these areas, although it is acknowledged that the vested commercial interests in the research and its outcomes adds a major complicating factor to the progression of science, the use of the knowledge, and the communications to the public. The appropriate government agencies have several important responsibilities in this matter: to support further research in the area (especially clinical research); to clearly communicate the state of knowledge and judged risks to the public, and; to take specific actions to promote and protect the health of the public, where appropriate.

The first two actions are clearly warranted based on the evidence to date. In my opinion, however, I do not believe there is sufficient evidence to warrant the government agencies taking further specific public health actions such as changing dietary recommendations, requiring labelling of products containing A1 β-casein, or encouraging changes in the dairy herd composition in order to promote and protect the health of the population. There is a requirement to monitor the health claims being made for A2 milk to ensure that they comply with existing regulations.

Those involved in the dairy and associated industries have to make their own judgements about strategies under their control such as changing dairy herd composition. These decisions will undoubtedly be made on a commercial basis. Changing dairy herds to more A2 producing cows may significantly improve public health, if the A1/A2 hypothesis is proved correct, and it is highly unlikely to do harm.

As a matter of individual choice, people may wish to reduce or remove A1 β-casein from their diet (or their children's diet) as a precautionary measure. This may be particularly relevant for those individuals who have or are at risk

of the diseases mentioned (type 1 diabetes, coronary heart disease, autism and schizophrenia). However, they should do so knowing that there is substantial uncertainty about the benefits of such an approach.

1. BACKGROUND

1.1 Purpose of this document

The potential role of A1 and A2 β-casein in health and disease (especially type 1 diabetes and cardiovascular diseases) is currently very topical and carries with it substantial public health and commercial implications. The debate around the issue has involved scientific research, media reports, expert commentary, claims and counter claims by the commercial interests involved, lawsuits, and public discussion.   A heated debate about the science and its public health implications when commercial interests are heavily involved makes for a very difficult environment for the government agencies responsible for promoting and protecting public health. Independent evaluation of the level of evidence and the health implications is needed to support those agencies in their communications to the public and their use of the regulatory and other processes available to them. In addition, new scientific information continues to be published and the implications of this new evidence need to be incorporated into public health decisions. This document has been prepared for the New Zealand Food Safety Authority to help it in this regard.

The report has 2 components:

a) A descriptive list of available research in the area of A1 β-casein and A2 β-casein in milk produced through:

  – a literature search; and

  – information that might be provided to NZFSA in response to requests for such information from stakeholders; and

  – any other means that will enhance the comprehensiveness and completeness of the material presented.

b) A critical analysis and evaluation of the research identified, taking into account the related areas of:

  – Nutrition

  – Human health

- Cardiovascular disease
- Diabetes
- Neurological disorders such as schizophrenia and autism.

The literature search strategies employed were wider than A1 and A2 β-casein and included casein in general because of the relevance of such studies.

1.2 A1 and A2 β casein

Casein represents about 80% of total protein in cows' milk, about 30-35% of which is β-casein (1). Within β-casein, there are a number of variants which are genetically determined. A1, A2 and B are the most common variants. A1 and, to a lesser extent, B have been implicated in the literature in relation to human disease. A1 and B β-casein have a histidine at position 67 that allows an enzymatic cleavage to occur releasing a 7 amino acid called 'β casomorphin 7' (βCM-7) (2). The A2 variant has a proline at position 67 so that βCM-7 is not released. βCM-7 has opioid properties and has a wide variety of potential effects in the body, including immune suppressant activity. These may be important in any potential etiological effects of A1 β-casein on disease processes, although other mechanisms are also possible and far more research is needed in this area.

1.3 Milk, early nutrition, and human health

Early nutrition has important health consequences for both childhood and adulthood. Cows' milk is a basic food for infants and children and a common food for adults in western countries. The health conditions that have been postulated to be affected by the A1/A2 β-casein content of milk are important and are reasonably common. Therefore, if the composition of milk is a causative or protective factor for these conditions, it has major public health implications.

1.4 Methodology

The PubMed database was searched for "A1 casein" as a key word and exploded MeSH headings (1) "Diabetes Mellitus" (references, n = 10), (2) "Cardiovascular Disease" (n = 6), (3) "Arteriosclerosis" (n = 0), (4) "Autistic Disorders" (n = 0), and (5) "Schizophrenia" (n = 0). Because of the low number of papers and potential relevance of research on casein that was not captured in the above strategy, the MeSH heading "Caseins" was also searched with (1) "Diabetes Mellitus" (n = 68), (2) "Cardiovascular Disease" (n = 115), (3) "arteriosclerosis" (n = 41), (4) "Autistic Disorders" (n = 7), and (5) "Schizophrenia" (n = 1). The search years were 1966 to May 2003. The results from the first search strategies were a subset of the second search strategies. Only English language papers were included in the electronic search although some relevant foreign language papers with English abstracts were also obtained from other sources. Additional papers were sought from the reference lists of the obtained articles and from other sources. Other papers that became available after this date and before May 2004 while the initial report was under peer review were also included.

Papers that presented data that were relevant to the project aims (above) were identified, analysed and summarised in a table. Many of the papers that were sourced under the 'caseins' MeSH heading were animal studies where a casein-based diet was used as the comparison diet for feeding studies that were testing the effects of other diets (such as soy-based diets). These studies were not included. Some other studies were included that did not examine A1 or A2 β-casein specifically but provided some background to research that has examined the specific health conditions in relation to casein in general, milk proteins, or milk.

The overall results are discussed below under the headings of 'type 1 diabetes', 'cardiovascular diseases' (incorporates arteriosclerosis) and 'neurological disorders' (incorporates schizophrenia and autism).

Some review and opinion articles have also been used to provide overview comments. A table of the papers reviewed is included in appendix 1.

2. DIABETES MELLITUS TYPE 1

2.1   Etiology and hypotheses

Diabetes mellitus type 1 (DM-1) develops as a result of the destruction of the insulin-secreting pancreatic β cells. This is mediated by an autoimmune process where T cells are thought to play a major role. The T cell immune response is also known as a 'cell-mediated' immune response as distinct from an 'antibody' or 'humoral' immune response which is largely mediated by B cells. The loss of insulin secretion results in clinical diabetes and a lifetime dependence on insulin injections. There is agreement that the process involves an exogenous agent or agents (eg food components, viruses) that trigger an immune response in genetically predisposed individuals. The hypothesis relevant to this review is that the A1 variant of β-casein is one such trigger. Genetic markers for susceptibility have been identified in the human leukocyte antigen (HLA) region and substantial research has been aimed at identifying the exogenous agents that trigger or facilitate the autoimmune processes. Exactly how the environmental trigger affects the immune systems has not been determined. There are certainly a number of antibodies that have been identified in people with DM-1 but these are seen in cross-sectional studies and therefore may simply be epiphenomena rather than causal.

Positive associations between DM-1 incidence and milk consumption and negative associations with breastfeeding have been shown in epidemiological studies (3), but not all studies have found these relationships (4). Evidence that milk consumption is related to DM-1 has been summarised by Schrezenmeir and Jagla (5) and Pozzilli (6). Table 1 shows the combined 'pros' and 'cons' for a relationship between milk consumption and DM-1.

Table 1: Evidence for and against a role of milk in the development of type 1 diabetes (adapted from Schrezenmeir and Jagla 2000, Pozzilli 1999)

Pro	Con
Increased incidence of DM-1 in children not breastfed or <4 month after birth	No correlation between DM-1 and frequency and duration of breastfeeding
Association between diabetes prevalence and cows' milk consumption	DM-1 incidence was increased after introduction of solid food before the 4th month
Presence of antibodies against BSA (bovine serum albumin) and ABBOS peptide (residue of BSA)	Antibodies to BSA are not specific for type 1 diabetes (also other autoimmune diseases)
Structural homology of β-casein with p69, carboxypeptidase and glucose transporter GLUT-2 of islet cells	Relationship between anti-BSA, anti-ABBOS and anti-p69/ICA69 is questionable – epiphenomenon?
Presence of autoantibodies binding β-casein A1 in (pre)diabetics	Anti-BSA is only a reflection of disturbances in foreign/own recognition
Increased levels of IgA antibodies to BLG (and BSA) and independent association of IgA antibodies to BLG (but not to BSA) and cow's milk with the risk of DM-1	Injection of the BSA peptide ABBOS into young mice even reduces diabetes incidence
Cows' milk diet increased manifestation of autoimmune diabetes in animal models	In some studies milk/BSA did not increase diabetes incidence in NOD mice
Immunisation of NOD mice (but not healthy strains) with BSA or p69 generated cross-reactive T-cell responses to T-cell epitope Tep p69 and ABBOS (but not albumin)	Bovine IgG has shown even to be protective in NOD mice
β-casein and β-casomorphin induce diabetes in NOD mice	Meat and soy protein also associated with increased the manifestation of DM-1
Only from β-casein A1, not from β-casein A2, casomorphin-7 is released which stimulates macrophages; diabetes induction by β-casein A1 is inhibited by naloxone, an opiate antagonist	Skim milk powder is variably and on average only mildly diabetogenic in the BBdp rat and in the NOD mouse. Plant constituents, particularly from wheat and soy, are more potent and consistent diabetogens
Skim milk powder can be diabetogenic in diabetes-prone BB rats	Patients with DM-1 have raised levels of antibodies against many diverse antigens; there is nothing unique about cow's milk antigens
Low consumption of A1 β-casein and low incidence of DM-1 among Icelanders compared to other genetically related Nordic populations	The possible cross reactivity between β-casein and BSA with islet antigens is not unique; antigens of several viruses and myobacteria also cross-react with other β-cell antigens

Further support for an effect of milk exposure comes from a carefully conducted case-control study in Finland where DM-1 cases (n=33) had a greater likelihood of a high consumption of milk (odds ratio 5.37, 95% CI 1.6 to 18.4) compared to controls (n=254) (7). There were no differences in the age at introduction of supplementary milk feeding.

The 'milk hypothesis' was further refined to the A1 and B components of β-casein in milk and this was supported by ecological data showing better correlations for A1 and B β-casein than for milk protein (2). Mechanistically, the concept of 'cross-reactivity' between antigens in cows' milk and antigens on the pancreatic β-cell is appealing and has been proposed by several investigators (8). More recently, a more elaborate potential mechanism has been proposed (2). It is hypothesized that the βCM-7 cleaved from A1 β-casein has opioid characteristics which suppress the body's immune surveillance or responses to antigenic agents such as enteroviruses or endogenous retroviruses which then damage the pancreatic β-cells.

2.2 Ecological studies

The best ecological study of DM-1 and A1/A2 β-casein was that published by Laugesen and Elliott (9). The other ecological studies (2, 10, 11) were earlier versions of the same analytical approach with fewer countries included. Laugesen and Elliott (9) had 19 countries in their dataset, a wide range of A1 β-casein consumption, and a wide variety of analyses of different combinations of casein fractions. Overall, the study has been very carefully undertaken and the strengths and limitations of this type of study are well set out in the discussion.

Results of note were:
- The highest correlation (r=0.92) with DM-1 incidence was found for A1 β-casein in milk and cream

- The addition of B and C β-casein slightly weakened the relationship whereas, adding B β-casein in the earlier study by Elliott et al (2) strengthened it
- The A2 β-casein relationship was positive and moderately strong (r=0.47)
- The relationship was substantially weaker for A1 β-casein in cheese (r=0.46) and for total milk protein (r=0.68 and 0.64)

Interpretation of these results needs to be made in light of the following factors:

- A correlation coefficient (r value) over 0.90 is extremely high for such ecological studies. The correlation of 0.96 means that 92% (ie R^2=0.92) of the variance in DM-1 incidence can be explained by the A1 β casein intake from milk and cream per capita. This is a very high 'goodness-of-fit' of the data.
- The correlation coefficient is very dependent on the range of the variables tested. There was nearly 300-fold range in DM-1 incidence rates and nearly a 6-fold range in A1 β-casein per capita. If a low correlation coefficient is found, it may just mean that the variation across countries is low (for example, if A1 β-casein was truly an etiological factor in DM-1 development, ecological studies like this one may not detect any relationship if all countries had very similar intakes of A1 β-casein or very similar incidence rates for DM-1).
- High correlation coefficients can also be obtained when there are one or two outlying points. This was clearly demonstrated by McLachlan in the relationship between ischaemic heart disease mortality and A1 β-casein consumption in his letter in response to the Laugesen and Elliott paper (12). When one data point (Netherlands) was deleted and one (NZ) was recalculated, the R^2 value went from virtually zero (0.0008) to 0.9. However, Laugesen and Elliott accounted for undue influence of one or two data points

in their paper by showing the scatter plot and analysing the results with various data points removed.

- Besides the goodness-of-fit criteria (r and R^2) for the associations between the variables, the strength of association (slope of the regression line) is also important to judge whether the relationship is strong or weak and thus relevant at a public health and clinical level. A 1% change in A1/capita was associated with a 1.3% change in DM-1 incidence. This is a strong relationship which, if it is cause and effect, is of real clinical and public health importance.

- Co-linearity between the variables (several variables closely related to each other) makes teasing apart the strongest factors a challenge and may give apparently contradictory results. For example, a high A2 β-casein (which is supposed to be protective) was associated with higher DM-1 incidence.

- Correlation does not mean causation. The classic fallacy from ecological studies is that the data are aggregated for a country and both variables may not apply to the same individuals. In other words, are the individuals who get DM-1 also the ones who had high A1 β-casein consumption? Spurious relationships can readily be seen in ecological studies. For example, the positive relationship between latitude and DM-1 incidence (noted in this paper) may just reflect the fact that northern European diets have more dairy products than southern European diets because the climate is more suited to dairy farming than cropping.

- The 'consumption' data are, in fact, 'apparent consumption' data based on food supply or disappearance information at a country level. As might be expected, these data are prone to many assumptions and errors. If these errors simply add 'random noise' to the estimates (ie the estimates are blunt but not biased in any particular direction), this makes the high correlations even more impressive. If, however, the assumptions and errors add non-random noise (ie estimates are biased), such bias may help to explain the high correlations. Having said that, there are no

obvious biases that would account for these high correlations. A
bias that is both unknown at the present stage and could, if
accounted for, make the published correlations insignificant is
possible, but unlikely.

- Other issues such as the accuracy and representativeness of the
  data are well covered in the paper's discussion.

- Because of the high potential for bias and the 'ecological fallacy',
  ecological studies are considered to be hypothesis-generating or
  hypothesis-supporting rather than hypothesis-confirming.

2.3   Clinical studies

A number of studies have examined the frequency of a variety of
antibodies in patients with DM-1 and controls. A summary of the case
control studies is included in appendix 1. The studies usually compare
patients with DM-1 (often newly-diagnosed) with normal controls or
patients with other autoimmume diseases such as thyroid disease.
Both cell-mediated (T cell) and humoral (antibody) immune systems
have been tested. Since the pancreatic β cell destruction is thought to
be T cell mediated, these studies may be of more relevance. The
studies that have used β-casein have been included in the review but
few have tested A1 or A2 variants specifically. The study by Padberg
et al (13) showed that antibodies against A1 β-casein are increased in
patients with DM-1. Immune responses to a variety of other antigens
(eg GAD, BSA, β lactoglobin, α-casein) were also tested. In general,
patients with DM-1 had higher levels of immune response to β casein,
but the overlap with controls was substantial and responses to other
antigens were also often increased. There is considerable debate
about whether this evidence of immune activity is linked to the etiology
of DM-1 or is a consequence of the disease process.

No longitudinal studies or feeding trials in humans have been reported
on the roles of A1 and A2 β-casein in the etiology of DM-1. Such

studies are possible (especially within genetically at-risk infants) and would contribute substantially to the evidence base.

2.4   Animal studies

Several feeding studies have been undertaken with a variety of animal models of DM-1. Often a pre-hydrolysed casein-based infant formula is used as a diabetes-**protective** diet (hydrolysing or breaking down of the casein molecule appears to remove any diabetogenic effect). A cereal-based diet (mainly wheat, corn and soybean) called NIH-07 is often used as a standard diabetes-promoting diet (14).

Two animal studies specifically tested the A1 / A2 fractions (15, 16). The more recent series of studies reported by Beales et al used 2 animal models and 9 different diets in 3 centres internationally. The most diabetogenic diet was a plant-based (mainly wheat), milk protein-free composition. This caused diabetes in 71% of NOD mice and 73% of BB rats. The base diet was either Prosobee (soy isolate) or Pregestamil (hydrolysed casein) and this resulted in diabetes rates between 17% and 39% in both animal models. Overall, the addition of 10% of either A1 β-casein or A2 β-casein made no difference to the diabetes rates except in the BB rat where Prosobee+A1 caused more diabetes than Prosobee+A2 (46% versus 19%, $p<0.05$).

These findings are in contrast to the earlier study reported by Elliott et al (16). Their findings were much more clear-cut showing a marked increase in diabetes incidence with the A1 compared to the A2 supplemented diet. This paper, however, lacked detail in methods and results making it difficult to interpret. The publication it appears in may not have been peer-reviewed and may not have required (or had room for) the level of detail normally seen in a peer-reviewed paper. The negative results of the multi-centre study (15) appear to be scientifically more robust (multi-centre, 2 animal models, blinded investigators,

standardised diets across centres, publication in a peer-reviewed journal) than this earlier positive study by Elliott et al (16).   Further studies will be needed to resolve the susceptibility of animal models for DM-1 to diets with variations in A1 / A2 β-casein content.

2.5   Summary and implications

The hypothesis that the exposure in infancy to A1 β-casein from cows' milk is etiologically associated with the development of DM-1 in genetically susceptible individuals is a fascinating one. Of the ecological studies, the latest one (9) is the most extensive and is very impressive. The correlation coefficients are very high for this type of study using these relatively blunt measurements (especially the food supply data). As mentioned above, the very wide variation in A1 exposure and DM-1 incidence rates will magnify the correlation coefficients. Residual bias and the classic 'ecological fallacy' mean that these studies cannot be accepted as proof of etiology, even with the high correlations. Having said that, however, sources of bias that could account for these high correlations are not apparent, and the ecological evidence demands more research.

The other human studies are all case-control studies of immune responsiveness and these are somewhat supportive of the A1/A2 hypothesis. However, a very valid alternative explanation for the observed immune reactivity to β-casein is that it is a *consequence* of the disease process rather than a cause of it.

The multi-centre animal study did not provide support for a role of A1 or A2 β-casein as either a causative or protective factor in the development of DM-1. It is also clear from other animal studies that other dietary candidates for triggering diabetes (such as wheat and other cereals) have a much more consistent evidence base.

The immediate (and undisputable) implication is that more research is urgently needed to investigate the A1/A2 hypothesis. Longitudinal and intervention studies in genetically at-risk infants will be very important in this regard. The sum of the evidence to date, in my opinion, does not constitute a sufficient evidence-base with which to:

- Make specific recommendations to the public about actions to take (in relation to milk exposure) to prevent DM-1
- Make policy-based decisions about reducing the exposure of infants to A1 milk
- Institute specific programs aimed at screening and intervening in high-risk families

The issue is, however, important enough for the appropriate government agencies to:

- Communicate the state of the current evidence to the public along with the levels of uncertainty and risks/benefits associated with infant feeding practices
- Actively promote high rates of initiation and continuation of breastfeeding for its well-established benefits as well as possible benefits such as protection from DM-1.
- Support further research in the area
- Monitor the health claims made for A2 milk and ensure that they are within the appropriate regulations for health claims for foods

The more difficult area to address in the face of this uncertainty is about advice to families, especially high risk families, who wish to take a highly precautionary approach. Concrete advice on breastfeeding and the use of infant formulas that use hydrolysed casein can be given. Thereafter, the level of avoidance of milk (or more specifically, milk with a high A1 content) and until what age is a matter of individual choice for those families because the scientific evidence does not provide a strong enough basis for advice.

3. CARDIOVASCULAR DISEASES

3.1 Etiology and hypotheses

The major cardiovascular diseases (CVD) are ischaemic (or coronary) heart disease (IHD) and stroke. Both have common underlying processes of atherosclerosis (deposition over many years of cholesterol and other material under the intimal lining of arteries) and thrombosis (acute clot inside the atherosclerotic artery). The classical risk factors for CVD are high total and LDL cholesterol levels, high blood pressure, smoking and physical inactivity. A large number of other risk factors for CVD have also been identified such as elevated homocysteine and lipoprotein (a) levels. Diet plays a major role in influencing serum cholesterol, blood pressure, homocysteine, and a large number of other factors (such as anti-oxidant status). There are undoubtedly many other ways that diet affects CVD risk that have yet to be discovered.

The risk factors for IHD and stroke are slightly different (despite similar underlying pathological processes), for example serum cholesterol levels appear to be more important for IHD and high blood pressure appears to be more important for stroke. IHD is a much bigger contributor to overall mortality than stroke. Mortality and incidence rates IHD are decreasing in New Zealand from a peak in the late 1960s.

The component of dairy products that has been repeatedly and convincingly demonstrated to increase the atherogenic LDL cholesterol levels is its saturated fat content. The New Zealand consumption of saturated fat is amongst the highest in the world and a large proportion of this is of dairy origin(17, 18). Reducing the consumption of saturated fat in New Zealand remains a high public health priority and switching from full-fat to reduced-fat dairy products is an important message in this regard.

The contention of the A1/A2 hypothesis is that a high intake of A1 β-casein is a further risk factor for IHD. There is some evidence that animal proteins are more cholesterolemic and atherogenic than plant proteins (19). Several animal models (rabbits, monkeys, mice) have shown that a high casein consumption promotes atherosclerosis (cited in (20)). While there are potential pathways linking A1 β-casein to IHD, no coherent mechanistic process has been demonstrated.

3.2 Ecological studies

The best ecological study on the relationship between A1 β-casein and IHD was the one published by Laugesen and Elliott (9). The strongest relationship with IHD mortality across 21 countries was for A1 β-casein/capita in milk and cream (r=0.76-0.81). This is a very strong relationship especially considering the crude nature of many of the measurements (food balance sheets and national mortality statistics). The wide variation in A1 consumption (13-fold) across countries again increases the chance of finding statistical relationships compared to those risk factors with a very low variation (eg tobacco consumption varied less than 2-fold). The highly multi-factorial nature of IHD makes such strong correlations even more surprising.

A 1% change in A1/capita consumption was associated with a 0.57% reduction in IHD in 1995. This is a strong effect size and is relevant at a clinical and public health level.

Having noted the strength and goodness-of-fit of the relationship, however, all the same caveats apply to this relationship as noted above for DM-1 and such studies by themselves can not be the basis for formulating public health policy.

Paradoxically, the relationship between A2 β-casein consumption and IHD may also be positive (as it is with DM-1) but unfortunately this is

not reported in the paper. The co-linearity between the variables measured makes it difficult to tease out which factors are potential causal links and which are just epiphenomena. Therefore, if there was a positive relationship between A2 β-casein consumption and IHD, it could carry a number of theoretical interpretations: It may be a simple co-linearity with the real causal agent (eg saturated fat intake or A1 β-casein intake), or; contrary to the A1 hypothesis, A2 could be causally involved in the development of IHD, or; there is no true relationship between a high A2 intake and IHD death because they occur in different individuals and the relationship only applies to the aggregated country data not the individual data.

The fact that no association was found between IHD and some of the well-established conventional atherogenic risk factors such as tobacco consumption and saturated fat consumption, only serves to highlight the shortcomings of these ecological analyses because their role in the etiology of IHD has been well established elsewhere.

The paper by McLachlan (11) was an earlier version of the same ecological analyses. The paper by Hill et al (21) demonstrated that the relationships between milk protein consumption and IHD mortality rates were much stronger in the 1970s (r~0.70-0.76) than the 1990s (r~0.01-0.30) and that the choice for a lag time for the analyses is not critical. The latest relationships (1995) in this analysis were very weak and (eg r=-0.06, 5 year lag) compared to Laugesen and Elliott's estimates with the same lag period (r=0.60, p<0.01). These major discrepancies between the two studies in the correlation values they report have not been resolved.

3.3   Clinical evidence

No clinical studies were found that have tested the relationships between A1 or A2 β-casein consumption and IHD or its risk factors. Two short-term trials tested the effects of casein-based or soy-based

diets (22). One study reported benefits for the soy-based diet on some parameters (reduced LDL cholesterol and homocysteine levels) and the other found one parameter improved with casein (reduced lipoprotein (a)) and one improved with soy (increased HDL). A number of parameters were no different between the diets. These studies indicate that the type of protein may have important effects on CVD risk factors but there was little consistency in the results and they were not specific for A1 or A2. The available clinical studies, therefore, contribute very little to the evidence for or against the hypothesis on A1 β-casein consumption and risk of IHD.

3.4   Animal evidence

Several animal studies have used casein-based diets in feeding studies but only one paper (20) has addressed the specific effects of A1 and A2 β-casein. The animal model for atherosclerosis was the rabbit which develops cholesterol deposits under the intimal lining of arteries in response to a diet which is usually very high in cholesterol. The fatty streaks thus produced in the main arteries resemble those seen in humans, although the pathological processes may be different between the rabbit model of 'cholesterol overload' and human atherosclerosis. Large amounts of A1 or A2 β-casein (or whey as the control diet) were fed to groups of rabbits with or without the high added cholesterol to the diet. There were few differences in blood results (higher total cholesterol and LDL:HDL cholesterol on the A1 diet versus A2 diet in one of the dietary conditions – 10% β-casein with no added dietary cholesterol). The anatomical measures showed no differences in the balloon-damaged arteries. In the non-damaged arteries, the A1 group showed increased area of fatty streaks in one of 4 comparisons with A2 and increased intimal thickening in 4/4 comparisons. There are some concerns about the methodology in this trial – the sample sizes were small (6 per group); it was not stated that a single or a blinded operator did the measurements, and; the number of aortic sections is small. This study provides some support for the

A1/A2 hypothesis for IHD, but it is not substantial because it is a single study in an animal model, many of the outcomes were no different between the A1 and the A2 dietary conditions, and there are several methodological concerns. Translation of results from animal models to humans is always problematic.

3.5 Summary and implications

The hypothesis that a high consumption of A1 β-casein promotes atherosclerosis and IHD is very interesting, although the biological plausibility of a causal relationship cannot be assessed because the potential mechanisms have not been well defined. Irrespective of putative pathways, is the empirical evidence sufficient to warrant more than a statement about the need for more research? The specific evidence comes from a strong relationship in an ecological study and some potentially supportive findings in one animal study. Many potential biases exist in these studies and, in my opinion, there is currently insufficient evidence to support changing the food-based dietary guidelines for the population, changing dietary advice to high-risk individuals, or implementing public health policies and programs to reduce A1 β-casein consumption as a preventive measure for IHD.

CVD is a multi-factorial disease and there are many major risk factors that are supported by a wealth of evidence that need to be addressed. For example, there is substantial room for improvement in New Zealand to further decrease smoking, saturated fat intake, and salt intake and further increase physical activity, fruit and vegetable consumption, and omega-3 fatty acid consumption. These changes would substantially reduce the risk of CVD for individuals and populations. One of the risks of focussing on new potential risk factors with marginal evidence is that the important other major risk factors with good evidence are forgotten.

4.   NEUROLOGICAL DISORDERS

4.1   Background

The hypothesis that links neurological disorders such as schizophrenia and autism to A1 β-casein is that, in genetically susceptible individuals, dietary components like casein and gluten are cleaved in the gut to produce peptide fragments with opioid characteristics (gluteomorphines and casomorphines) (23). These compounds enter the circulation, cross the blood:brain barrier and influence neurological functioning. A marker of diet-responsivity is said to be abnormal urinary peptide excretion(23). The hypothesis is usually stated as an etiological relationship but, if there is a true relationship, it could equally be a dietary aggravation of an existing condition with unrelated etiology (in the same way that dietary sodium aggravates but does not cause heart failure).

There are very few studies in this area and none examined A1 versus A2 β-casein specifically. Almost all dietary manipulations combined casein-free with gluten-free diet.

4.2   Schizophrenia

One poorly controlled case-control study found elevated IgA antibodies to casein as well as gliaden, gluten and β-lactoglobulin in schizophrenic patients(24).

4.3   Autism

Eight trials of casein-free diets in people with autism have been published (23, 25). The trials have generally been of poor scientific design with no control group or blinding of measurements. Seven of the trials were uncritically reviewed by Knivsberg et al (25) although taking the study biases into account, they were suggestive of an improvement in several autistic behaviours and functioning with casein-free, gluten-free diets. The best study was the most recent one (23) which had a better design

with blinded assessments where possible and a random allocation to diet or no diet. The majority of the measurements showed significant improvements on the diet (casein-free, gluten-free).

4.4 Summary and implications

The available evidence is suggestive of a role of reducing the casein and gluten in the diets of people with autism to improve the autistic behaviours and overall functioning of the individual. Further research is needed. The evidence is not strong enough for clear dietary recommendations to be made for people with autism and schizophrenia. Decisions to trial a casein-free, gluten-free diet should be made between the individuals, their carers, and their doctors.

5. OVERALL CONCLUSIONS

The hypothesis that a high intake of milk containing A1 β-casein promotes conditions as heterogeneous as DM-1, IHD, schizophrenia and autism is intriguing and potentially important. There is some very suggestive evidence from ecological studies for DM-1 and IHD, and there is certainly a possibility that the A1/A2 composition of milk is a factor in the etiology of these conditions. However, this hypothesis has yet to be backed by good human trials. The evidence in relation to autism comes mainly from poorly controlled clinical trials of gluten-free, casein-free diets where some improvement is noted in the autism characteristics and behaviours. The evidence in relation to schizophrenia is very minimal.

In my opinion, the warranted actions at present by the relevant government agencies involve:

- Funding further research, especially clinical research
- Communicating the current evidence and the uncertainty to the public about the A1/A2 hypothesis and its implications for milk consumption
- Monitoring new evidence as it is published and reviewing public health action
- Monitoring the claims being made to the public about health benefits of A2 milk and ensuring that they are within the food claims regulations

I do not believe that there is sufficient evidence as yet to warrant more specific population measures such as:

- Changing dietary advice to the general population
- Changing recommendations for specific dietary advice for those with (or at risk of) DM-1, IHD, autism or schizophrenia
- Requiring labelling of casein sub-type on dairy products
- Recommending changing dairy herds in order to improve public health outcomes

These recommendations are in line with those made by other highly qualified reviewers of this topic. Beaglehole and Jackson (26) recognised the potential

benefits to population health if the A1/A2 hypothesis is proved correct but note that a more pressing need in relation to the prevention of IHD is to implement those strategies which have been well-proven to be effective. Mann and Skeaff felt that health claims that A2 milk is protective of IHD were premature noting that similar claims had been made for vitamin E supplements based on early promising epidemiological evidence, but later well-conducted trials showed them to be of no benefit (27).

Those involved in the dairy and associated industries also have to make their own judgements about strategies under their control such as changing dairy herd composition. These would be based on their commercial assessments of the pros and cons of such a move. A New Zealand dairy herd that produced predominantly A2 milk would have no apparent negative health effects and could potentially have significant population health benefits if the A1/A2 hypothesis proves to be correct. Current claims for the health benefits of A2 milk need to remain restricted and comply with the appropriate regulations on food claims.

In discussion with their medical advisors, people with established IHD or at high risk of IHD, families with children at (genetically) high risk for DM-1, and families with autistic children may be motivated to reduce or eliminate their intake of A1 β-casein. This would be done as a precautionary approach (or possibly as a trial in the case of autism) in the face of substantial uncertainty about the potential benefits. The evidence does not support such dietary changes as a recommended clinical approach with a known likelihood of benefit.

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7. Appendix 1

Summary tables of the papers reviewed:

Type 1 diabetes mellitus

Cardiovascular diseases

Neurological disorders

Citation (ref No)	A1/A2 specific	Study Type	Summary
TYPE 1 DIABETES			
(9) Laugesen M & Elliott R. Ischeamic heart disease, type 1 diabetes, and cow milk A1 b-casein (2003). NZ Med J, 116; URL http://www.nzma.org.nz/ journal/116-1168/295/	Y	Ecol	The most complete ecological study to date (19 countries). Estimated A1/capita consumption from cow milk and cream supply (FAO database) and A1 β-casein fraction from a variety of sources. 19 countries excluding high milk importer/exporters, those with total health expenditure >US$1,000/capita and excluding countries not surveyed by WHO DiaMond Project of EURODIAB ACE. Milk and cream supply/capita was calculated from nutritional statistical databases at the FAO web site as milk protein/capita/day, excluded butter and cheese, goats and sheep's milk. Imported milk was adjusted for if over 20% of domestic milk usage. FAO food supply data were converted to nutritional measures using British food composition tables. Cow breed distribution was calculated from governmental animal census data, industry, or national breeding program data. β-casein fractions were estimated by breed from dairy science literature for 18 countries. Additionally, milk was tested from 11 countries. DM-1 incidence data from 1990-94 WHO DiaMond Project and EURODIAB ACE. Regional incidence results were averaged within each country. Over 75 foods and 100 nutritional food supply variables were tested for correlation against DM-1 in 1990-94. Average milk protein/capita varied fourfold across countries. A1 fraction of milk casein varied from 0.21 to 0.53. A1/capita from 0.4g/day to 3.0g/day. DM-1 rate varied nearly 300-fold from 0.13 to 36.5. DM-1 at age 0-14 correlated with milk and cream in the food supply, measured by milk protein/capita, particularly A1.

Strongest correlation was 0.92 for A1 β-casein/capita in milk and cream. A 1% difference in A1/capita was associated with a 1.3% difference in DM-1. Correlations were weaker for A1 & B β-casein and A1, B & C casein than for A1 alone. The putatively protective A2 β-casein was significantly positively correlated with DM-1 incidence. A 1% difference in A1/capita was associated with a 1.3% difference in DM-1 incidence.

Conclusions: A very strong ecological relationship between A1 consumption and DM-1 strongly supports the hypothesis, but cannot determine causality. |
| (10) Birgisdottir BE et al. Variation in consumption of cow milk proteins and lower incidence of type 1 diabetes in Iceland vs the other 4 Nordic countries (2002). Diab Nutr Metab, 15; 240-5 | Y | Ecol | Data on A1 + B β-casein from Elliot 1999. Strong positive relationship (n=5, r=0.90, p=0.037) between A1+B β-casein and DM-1 incidence in 5 Nordic countries. No relationships with consumption of other cow's milk proteins. **Conclusions:** As for Laugesen and Elliott (2003). |

TYPE 1 DIABETES Citation (ref No)	A1/A2 specific	Study Type	Summary
(11) McLachlan CNS. β-casein A[1], ischaemic heart disease mortality, and other illnesses (2001). Med Hypoth, 56; 262-272	Y	Ecol	Earlier version of the same ecological relationship between A1 β-casein consumption and DM-1 incidence (n=16 countries, R²=0.75). **Conclusions:** As for Laugesen and Elliott (2003).
(2) Elliott RB et al. Type 1 (insulin dependant) diabetes mellitus and cow milk: casein variant consumption (1999). Diabetologia, 42; 292-6	Y	Ecol	Original ecological study of A1 β-casein consumption and DM-1 incidence. Strongest relationship was for A1+B β-casein (n=9 countries, r=0.92, p=0.01). Relationships were stronger for A1 β-casein than for total milk proteins. **Conclusions:** As for Laugesen and Elliott (2003).
(3) Scott, F. Cow milk and insulin-dependant diabetes mellitus: Is there a relationship? (1990) Am J Clin Nutr, 51; 489-91.	N	Ecol	Milk-protein data from the OECD and breastfeeding prevalence data from WHO Nutrition Unit (1960's-70's). Significant positive relationship between consumption of unfermented milk protein and incidence of DM-1 (n=13 countries, r=0.86, p<0.01). Negative relationship between breastfeeding to 3 months and DM-1 incidence. **Conclusions:** Supports the hypothesis that breastfeeding may be protective and milk may promote DM-1, but no causality can be determined.
(28) Monetini L et al. Establishment of T cell lines to bovine b-casein and b-casein-derived epitopes in patients with type 1 diabetes (2003). J Endocrinol. 176; 143-50	N	CC	9 recent-onset people with DM-1, 7 controls (2 HLA-matched, 2 non HLA-matched, 3 first degree relatives). T cells specific to bovine β-casein were isolated from patients DM-1 but not controls. A potential for cross-reactivity with pancreatic β cell antigens was detected. **Conclusions:** T cells sensitised to β-casein are detectable in DM-1 and may cross react with the insulin-secreting cells in the pancreas. Uncertainty whether it is cause or effect.
(29) Banchuin N et al. Cell-mediated immune responses to GAD and β-casein in type 1 diabetes mellitus in Thailand (2002). Diab Res Clin Prac, 55; 237-45	N	CC	Cell-mediated (T cell) responses against β-casein were found in 18/38 (47.4%) patients with DM-1, 5/37 (13.5%) patients with DM-2, 1/43 (2.3%) controls using a lymphoproliferation assay. Cut-off levels used but substantial overlap in the absolute values. A similar pattern of responses was obtained using GAD (glutamic acid decarboxylase) – 76.3% in DM-1, 16.2% in DM-2,and 2.3% in controls. **Conclusions:** Higher level of cell mediated immune response to β-casein and GAD in DM-1 compared to DM-2 and controls. Uncertainty whether it is cause or effect.

TYPE 1 DIABETES Citation (ref No)	A1/A2 specific	Study Type	Summary
(30) Monetini L et al. Antibodies to bovine b-casein in diabetes and othe autoimmune diseases (2002). Horm Metab Res, 34; 450-4	N	CC	Increased levels of antibodies to bovine β-casein in DM-1 (n=71), coeliac disease (n=33), latent autoimmune diabetes (LADA, n=100) compared to age-matched controls. No difference in levels for autoimmune thyroid, multiple sclerosis, and lower levels in DM-2 compared to age-matched controls. Also confirmed previous data regarding high levels of IgG antibodies to bovine β-casein in patients with recent-set DM-1. **Conclusions:** The antibody (ie humoral) response to β-casein appears common in several autoimmune diseases. Large overlap in individual antibody levels between groups. Uncertainty whether it is cause or effect.
(31) Crino A et al. Intradermal skin test with diabetes specific antigens in patients with type 1 diabetes (2001). Clin Exp Immunol. 123, 382-6	N	CC	In vivo skin tests of antigens known to be recognised by T cells. Positive responses to β-casein in 2/16 recent onset DM-1, 0/16 autoimmune thyroid disease, 0/20 latent autoimmune diabetes. Greater in vitro stimulation of blood mononuclear cells by bovine β-casein in recent onset DM-1 versus normal controls (p<0.05) but large overlap in responses. **Conclusions:** Low sensitivity of this delayed-type hypersensitivity reaction to β-casein in DM-1.
(32) Monetini L et al. Bovine β-casein antibodies in breast- and bottle-fed infants: their relevance in Type 1 diabetes (2000) Diab Met Res Rev.17; 51-54	N	CC	Antibody response to bovine β-casein much higher in infants under 4months who were bottle-fed with cows milk (n=12) compared to exclusively breastfed infants (n=16), p<0.0001 (virtually no overlap in results). Antibodies also higher in prepubertal children with DM-1 (n=37) versus healthy controls exposed to dairy (n=31), P=0.03. **Conclusions:** Breastfeeding within the first 4 months of life prevents the generation of antibody response to bovine β-casein despite mothers' consumption of cow's milk during breastfeeding period.
(33) Thorsdottir I et al. Different β-casein fractions in Icelandic versus Scandinavian cow's milk may influence diabetogenicity of cow's milk in infancy and explain low incidence of IDDM in Iceland (2000). Pediatrics, 106; 719-24	Y (national milk supply only)	CC	Retrospective case control study of 55 DM-1 and 165 randomly selected, matched controls born in Iceland during 16yr period. No significant difference in frequency and duration of breastfeeding or the first introduction of cow's milk products. Note the low A1 and B β-casein in Icelandic milk compared to Scandinavian milk and that the incidence of DM-1 in Iceland is less than half that in other Nordic countries despite similar genetic backgrounds. **Conclusions:** A negative case-control study in one country with low A1 β-casein is not supportive of the A1 β-casein hypothesis but may not be inconsistent with the hypothesis if there is a relatively uniform exposure with genetic background creating the variance.
(13) Padberg S et al. The significance of A1 and A2 antibodies against β-casein in type-1 diabetes mellitus. (1997). Dtsch Med Wochenschr, 124; 1518-21	Y	CC	Antibodies against A1 and A2 β-casein were tested in patients with DM-1 (n=386), their siblings (n=477), their parents (n=287), their siblings and their parents (n=107) and healthy controls (n=107). Higher antibodies against A1 in DM-1 patients and their siblings, higher A2 antibodies in parents and controls. Higher antibody levels with younger age. **Conclusions:** Higher A1 antibodies in DM-1 and siblings may indicate differences in exposure (compared to controls), age (compared to parents), genetic response or other factors.

TYPE 1 DIABETES Citation (ref No)	A1/A2 specific	Study Type	Summary
(34) Sarugeri E et al. Cellular and humoral immunity against cow's milk proteins in type 1 diabetes (1999). J. Autoimmunity, 13; 365-73	N	CC	Case control study comparing newly diagnosed DM-1 with controls (unspecified sampling, not well matched). Cell-mediated responses (T cell): No differences in responses to β-casein, α-casein, β lactoglobin and BSA in DM-1 (n=23) and controls (n=22). Humoral (antibody) responses: higher antibody levels for β-casein, α-casein in DM-1 (n=59) and controls (n=52). **Conclusions:** Immune responses to cow's milk are not limited to DM-1 patients and are not solely against β-casein
(35) Ellis TM et al. Cellular immune responses to b-casein: elevated in but not specific for individuals with type 1 diabetes mellitus (1997). Diabetologia, 41; 731-5	N	CC	Case control study of cell mediated immune response to β-casein. Higher responses in DM-1 (n=71) compared to controls (n=10) but not compared to relatives (n=29) with none of the common DM-1 related antibodies (IAA and ICA). **Conclusions:** The significance of anti β-casein cell-mediated immunity as a specific factor in the pathogenesis of DM-1 remains unclear.
(1) Cavallo, MG and Fava, D. Cell-mediated immune response to beta casein in recent-onset insulin-dependant diabetes: Implication for disease pathogenesis (1996). Lancet, 348; 926-9.	N	CC	Earlier case control study of T cell response to β-casein. Higher proportion of positive responses in DM-1 (24/47, 51%) than in autoimmune thyroid disease (0/10) and controls (1/36, 2.7%) p<0.0001. **Conclusions:** High T-cell response to β-casein inDM-1. Uncertainty whether it is cause or effect.
(36) Elliott, RB et al Diabetes and cows' milk (1996) Lancet, 348, 1657 (letter).	N	CC	Case control study of antibodies against A1 β-casein and A2 β-casein in Germany (significant for A1 and A2, n=83), New Zealand (significant for A1, n=40), and Sardinia (not significant, n=57) between cases and controls. Lowest levels were in Sardinia which paradoxically has a much higher incidence of DM-1 than Germany or NZ. **Conclusions:** Some support for a humoral (antibody) response against β-casein (A1 and A2) but not in Sardinia. Uncertainty whether it is cause or effect.
(37) Cavallo MG et al. Diabetes and cows' milk (1996). Lancet, 348; 1655 (letter).	N	CC	Antibodies against β-casein are present in 20/54 (37%) of DM-1 patients compared to 3/53 (6%) of controls. Antibodies against BSA also higher (11% versus 4%). **Conclusions:** Antibodies against β-casein and other targets higher in DM-1. Uncertainty whether it is cause or effect.

TYPE 1 DIABETES Citation (ref No)	A1/A2 specific	Study Type	Summary
(15) Beales PE et al. A multi-centre, blinded international trial of the effect of A1 and A2 b-casein variants on diabetes incidence in two rodent models of spontaneous type 1 diabetes (2002). Diabetologia, 45; 1240-6	Y	Animal	Multi-centre study of 9 diets in 2 animal models of DM-1 in 3 countries with blinded assessment of outcomes. 3 labs – NZ (NOD mice), Canada (BB rats) & UK (NOD mice). Diets based on Pregestimil (PG) or ProSobee (PS) either alone or with purified fractions of either (1) whole casein (WC), (2) A1 β-casein or (3) A2 β-casein at 10%. A milk-free, wheat-predominant control diet was also included. Animals fed from weaning up to 150 (rats) or 250 (mice) days. NZ site confounded by an infection. All locations reported the highest incidence of diabetes in the milk-free wheat diet. PS & PG diets were protective except in PG+WC in UK where diabetes incidence was similar to control diet. A1 was slightly more diabetogenic in the Canadian trial, but only in the PS diet. UK mice on A2 β-casein still developed diabetes, unlike previous NZ trials. Overall, A1 & A2 diets were <u>protective</u> compared to control diets and varied little in diabetes-promoting capacity. **Conclusions:** These studies were not supportive of previous NZ studies showing A1 β-casein to be diabetogenic (Elliott 1997). Milk caseins are unlikely to be exclusive promoters of DM-1 (wheat may be more important). In these studies, the investigators were blinded which was apparently not the case in previous trials (mentioned by Scott FW and Kolb H, NZMJ 2003; 116 No 1170).
(16) Elliott RB, Wasmuth HE, Bibby NJ, Hill JP. The role of β-casein variants in the induction of insulin-dependent diabetes in the non-obese diabetic mouse and humans. (1997). International Dairy Federation, Brussels. pp445-453	Y	Animal	The incidence rates of diabetes in the NOD mouse model were determined on various diets: chow, 37%; Prosobee, 0%; Prosobee + 10% 'Samoan diet', 12%; Prosobee + 10% 'Finnish diet', 36%. Prosobee is a soy-based infant formula. The specifications of what constituted a 'Samoan diet' and a 'Finnish diet' were not presented which is one of the main drawbacks of this paper which does not appear to be in a peer-reviewed publication. Other feeding studies showed a marked effect on diabetes incidence with diets of added A1 β-casein (47%) versus A2 β-casein (0%). Hydrolysing the casein markedly reduced the diabetes incidence (from 28% to 2%) NOD mice fed A1 β-casein did not develop diabetes if naloxone (opiate antagonist) was also given but no data were shown for this. **Conclusions:** These studies show apparently clear-cut effects of A1 promoting diabetes and A2 providing protection. Regular chow was also diabetogenic. The methods and results details within the paper are less than would be expected from a peer-reviewed journal and this makes interpretation difficult. The data on the so-called 'Samoan' and 'Finnish' diets are uninterpretable without more information.

Ecol = Ecological Study
CC = Case Control Study
Animal = Animal Study

CARDIOVASCULAR DISEASE Citation (ref No)	A1/A2 specific	Study Type	Summary
(9) Laugesen M & Elliott R. Ischaemic heart disease, type 1 diabetes, and cow milk A1 b-casein (2003). NZ Med J, 116; URL http://www.nzma.org.nz/journal/116-1168/295/	Y	Ecol	The most complete ecological study to date (21 countries). Estimated A1/capita consumption from cow milk and cream supply (FAO database) and A1 β-casein fraction from a variety of sources. 21 countries excluding high milk importer/exporters, those with total health expenditure >US$1,000/capita. Milk and cream supply/capita was calculated from nutritional statistical databases at the FAO web site as milk protein/capita/day, excluded butter and cheese, goats and sheep's milk. Imported milk was adjusted for if over 20% of domestic milk usage. FAO food supply data were converted to nutritional measures using British food composition tables. Cow breed distribution was calculated from governmental animal census data, industry, or national breeding program data. β-casein fractions were estimated by breed from dairy science literature for 18 countries. Additionally, milk was tested from 11 countries. CVD mortality data were from WHO database (plus Channels Is Departments of Health). A lag of 5 years was included in the correlation analyses between food supply and CVD mortality. Average milk protein/capita varied 4-fold across countries. A1/capita varied 13-fold from 0.3g/day to 3.8g/day. Tobacco consumption varied less than 2-fold and saturated fat by 2.4-fold. IHD mortality rate varied five fold from 25 to 131 per 100,000.

IHD correlated most strongly with A1/capita supply in milk and cream for 1980,1985, 1990 and 1995 (r=0.76 to 0.81). Correlations were weaker if A1 supply from cheese was added, or if total milk proteins were used. A 1% difference in A1/capita consumption was associated with a 0.57% difference in IHD mortality in 1995. Interestingly, the correlations with A2/capita were not given (note the positive correlations between A2/capita and DM-1). A variety of other potential risk factors or protective factors were tested and these were either weaker than the correlation for A2 or were not significant (in particular tobacco and saturated fat were not significantly related to IHD mortality). A1/capita was the only significant variable in a multivariate analysis for 1995 IHD mortality.

Conclusions: For all the crudeness of the primary data, correlations of 0.7-0.8 are very strong and support the A1 hypothesis for IHD but such ecological relationships cannot determine causality. The strength of the relationships with A1 and the weakness with other more classical risk factors will be strongly affected by the range of the variables being tested. It is unfortunate that relationships with A2 β-casein were not published. |

CARDIOVASCULAR DISEASE Citation (ref No)	A1/A2 specific	Study Type	Summary
(11) McLachlan CNS. β-casein A[1], ischaemic heart disease mortality, and other illnesses (2001). Med Hypoth, 56; 262-272	Y	Ecol	Earlier version of the same ecological relationship between A1 β-casein consumption and IHD mortality (n=17 countries, R=0.93). A significant correlation was also presented for A1β-casein consumption and IHD mortality in 8 German counties (r=0.81). Northern Ireland has an IHD mortality about 3-fold higher than France without obvious differences in classical risk factors. The A1 β-casein consumption in Northern Ireland is also about 3-fold higher. **Conclusions:** As for Laugesen and Elliott (2003)
(21) Hill JP et al. Milk and consumer health: a review of the evidence for a relationship between the consumption of β-casein A1 with heart disease and insulin-dependent diabetes mellitus. (2002) Proc NZ Soc Animal Prod; 62:111-4	N	Ecol	A review paper on Aβ-casein and IHD and DM-1. New data presented on the time trends and lag periods in the relationship between milk protein consumption and IHD mortality in 40 countries. Much higher correlations for IHD deaths in the 1970s (~0.70-0.75) than the 1980s (~0.30-0.70) and the 1990's (~0.01-0.30) with milk protein consumption. There was very little effect of the lag period chosen between exposure (milk protein consumption) and outcome (IHD mortality). By the mid 1990s, the correlations were generally r<0.05. **Conclusions:** Very marked reductions in relationships between IHD and milk protein over time could be due to many factors that affect IHD mortality rates (such as changes in classical risk factors and improvements in medical treatment) or factors in the milk (such as reducing A1 content). These data reduce concerns about the choice of lag period in other papers.
(38) Tonstad S et al. A comparison of the effects of 2 doses of soy protein or casein on serum lipids, serum lipoproteins, and plasma total homocysteine in hypercholesterolemic subjects. (2002). Am J Clin Nutr. 76; 78-84	N	RCT	RCT over 24 weeks of 2 doses of either soy or casein protein in 130 subjects with high cholesterol levels. Lower LDL cholesterol and homocysteine on the soy diet but no differences in lipoprotein (a), HDL cholesterol and triglycerides. **Conclusions:** The main aim was to test the effects of a soy diet and the casein diet was considered the comparison diet rather than the experimental diet. Some potential benefits of soy over casein in IHD risk factors.
(22) Nilausen K, Meinertz H. Lipoprotein (a) and dietary proteins: casein lowers lipoprotein (a) as compared with soy protein. (1999) Am J Clin Nutr 69;419-25	N	RCT	Cross-over design trial in 9 normocholesterolemic men of 3 diets: usual, food-based diet, liquid casein-based diet and liquid soy-based diet, each measured over 30 days. Compared to each other, the casein diet reduced lipoprotein (a) concentrations and the soy diet increased HDL cholesterol. LDL cholesterol and triglycerides were not different. **Conclusions:** Both soy and casein diets had different effects on lipoprotein levels, both of which may be beneficial in protecting against ischaemic heart disease.

CARDIOVASCULAR DISEASE Citation (ref No)	A1/A2 specific	Study Type	Summary
(20)Tailford KA et al. A casein variant in cow's milk is atherogenic. (2003) Atherosclerosis 170; 13-19	Y	Animal	The rabbit model of atherosclerosis usually requires a diet which is extremely high in cholesterol, producing a cholesterol-laden foam cells that accumulate under the intimal lining of arteries. These fatty streaks are presumed to be the equivalent of the early stages of atherosclerosis in humans, although there is considerable debate about this.

In this study, 60 rabbits had their R carotid arteries de-epithelialised with a balloon catheter and then randomised into 10 groups of 6 rabbits each for study: 2 control whey diets (with and without high dietary cholesterol), 4 A1 diets or 4 A2 diets (different concentrations of A1 or A2 and with/without high dietary cholesterol.

Blood tests: 2 results were statistically significant between A1 and A2, (higher total and LDL cholesterol on A1 diet under conditions of no added dietary cholesterol). No differences in triglyceride or homocysteine levels under any conditions. No differences in lipids with the high dietary cholesterol conditions. Anatomical results: No differences between A1 and A2 in the damaged carotid arteries (measured as the intima:media ratio) under any of the dietary conditions. In the non-damaged area (aortic arch), the percentage of the arterial surface affected by fatty streaks were higher the A1 group compared to the A2 group in one out of 4 comparisons. The intima:media ratio in the aortic arch was higher in the A1 group compared to the A2 group in 4 out of the 4 comparisons. Summarised below are the statistically significant results in the A1 versus A2 comparisons under the 4 dietary conditions.

	Group 2 vs 3	Group 5 vs 6	Group 7 vs 8	Group 9 vs 10
Total serum cholesterol	P<0.05	NS	NS	NS
LDL:HDL cholesterol	P<0.05	NS	NS	NS
Triglycerides	NS	NS	NS	NS
Homocysteine	NS	NS	NS	NS
Aorta lesion % coverage	P<0.05	NS	NS	NS
Aortic arch intima:media	P<0.05	P<0.05	P<0.05	P<0.05
Carotid intima:media	NS	NS	NS	NS	

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			The methods as described raise a number of issues.
• The number of rabbits in each group was low (6). This means that one or two aberrant results could significantly skew the mean (eg in figure 2B the I:M ratio was obviously zero for all rabbits in groups 1 and 3 and conceivably for most rabbits in group 2 with one or two outliers). For such small numbers, actual data points should be shown.
• No mention is made if the operator conducting the measurements was the same person in all cases and whether he/she was blinded to the dietary condition.
• The sampling of the artery segments for analysis raises concerns about sampling errors. Only 3 sections were taken from the aortic arch (not specified how the sections were chosen), and cross-sectional measurements of intima:media thickness were made on an undisclosed number of slices. Contrast this with a similar study by Brasen et al (Atherosclerosis 2002; 163: 249-259) where a single blinded operator took 14 equal segments evenly distributed over the entire aorta and the intima:media ration was measured at 0.3mm intervals in each section giving a mean of 36 measurements for every cross-section and animal.

The source of funding for the project (apparently A2 Corporation) was not declared.
Conclusions: Some (a minority) of the blood measurements and anatomical changes measured were significantly more atherogenic in the A1-fed rabbits than the A2-fed rabbits. Differences were seen most often in the non-cholesterol supplemented diets (high cholesterol feeding usually needed in the rabbit model of atherosclerosis). Several concerns about the methodologies. The findings are mildly supportive of the A1 hypothesis of atherogenicity, but rabbits do not provide the ideal model for human atherosclerosis and, as is typical with many animal experiments, huge 'industrial' doses of the dietary substances being tested were used – in this study 20% of the diet was provided by the milk proteins being tested. Implications for humans need to be drawn from studies on humans. |

Ecol = Ecological Study
RCT = randomised controlled trial (human)
Animal = Animal Study
IHD = ischaemic heart disease

NEUROLOGICAL DISORDERS Citation (refNo)	A1/A2 specific	Study Type	Summary
(24) Reichelt KL & Landmark J. Specific IgA antibody increases in schizophrenia (1995). Biol Psychiatry. Vol. 37, 410-13	N	CC	IgA antibodies measured in 48 schizophrenics, 13 of whom were free from medication. The only controls were 13 health professionals age-matched to the 13 drug-free schizophrenics. 13/48 schizophrenics had anti-casein IgA levels above normal upper limits (higher than 1100 historical controls in another study). The drug-fee subgroup had a higher median anti-casein antibodies and higher frequency of abnormal values (5/13) compared with controls (frequency, 0/13). **Conclusions:** a poorly-controlled study, but suggesting high levels of antibodies to casein (as well as gliadin, gluten and β-lactoglobulin) in schizophrenics.
(23) Knivsberg AM, Reichelt KL, Hoien T, Nodland M. A randomised, controlled study of dietary intervention in autistic syndromes (2002). Nutr Neurosci. Vol 5, No. 4, 251-61	N	RCT	10 children with autism and abnormal urinary peptide excretion patterns were paired (matched for age, autism severity, cognitive level) then randomly assigned to be on gluten- and casein- free diet for 1 year. Single blind design with a battery of standardised tests before and after. The first set of tests was in the form of a questionnaire to parents (who were not blinded to the diet). In the diet group, significant improvements in 3/4 'attention' scales (aloofness, rituals, response to teaching – overall improvement vs controls, p<0.03), 4/6 'emotional and social factors' (peer relationships, anxiety, empathy, physical contact – overall improvement vs controls, p<0.004), 4/5 'communication' scales (non-verbal, eye contact, reaction when spoken to, language peculiarities – overall improvement vs controls, p<0.007), 2/4 'cognition' scales (expressed imagination, number of interests - overall improvement vs controls, p<0.02), and 1/5 'motor and sensory scales (extraordinary restlessness or passivity - overall no significant improvement vs controls, p=0.08). The second set of tests measured by a blinded investigator in the presence of the child's special educator (?blinding status). There were significant improvements in the diet groups compared to controls in autistic traits (p=0.001), non-verbal cognitive level (p=0.004), motor problems (p=0.04) but not linguistic age (p=0.37).

Conclusions: 1 year on a gluten- and casein- free diet appeared to improve multiple functions in autistic children with high urinary peptide excretion. The single blinding may have introduced some bias in the parent questionnaire, but the results are supported by the more objective testing. A well-conducted study with positive results. Gluten and casein were chosen for exclusion because of their potential for producing opioid breakdown products which may have neurological effects that impact on autistic patterns. The participants were therefore chosen on the basis of high urinary peptide excretion as a marker of high circulating opioid peptides. The specific roles of A1 and A2 β-casein were not tested. |

NEUROLOGICAL DISORDERS Citation (refNo)	A1/A2 specific	Study Type	Summary
(25) Knivsberg AM, Reichelt KL, Nodland M. Reports on dietary intervention in autistic disorders (2000). Nutr Neurosci. Vol 4, 25-37	N	Rev	Review paper on the evidence (up to 2000) for the hypothesis that autism is caused by genetic predisposition interacting dietary peptide overload, especially peptides with opioid activity (in the gut, gluten is broken down into gluteomorphines and caseins into caseomorphines). Seven trials of casein- and gluten- free diets were reviewed (total n=189). The quality of design was generally poor with only one trial having a randomly assigned control group and most being non-blinded. One trial of a casein-free diet rechallenged with casein or placebo capsules and showed deterioration in the casein group. 6/7 studies showed improvements on gluten-free and/or casein-free diets over periods from 3 months to 4 years.

Conclusions: The review of trials in this paper was not rigorous and the weaknesses and biases were not noted. Overall, the trials had marked methodological problems but their results largely support an improvement in autistic behaviours and functioning with a casein-free diet (usually in conjunction with a gluten-free diet). |

CC = Case control
RCT = Randomised controlled trial
Rev = Review (human)