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# REVIEW

# The A2 milk case: a critical review

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This review outlines a hypothesis that A1 one of the common variants of $\beta$-casein, a major protein in cows milk could facilitate the immunological processes that lead to type I diabetes (DM-I). It was subsequently suggested that A1 $\beta$-casein may also be a risk factor for coronary heart disease (CHD), based on between-country correlations of CHD mortality with estimated national consumption of A1 $\beta$-casein in a selected number of developed countries. A company, A2 Corporation was set up in New Zealand in the late 1990s to test cows and market milk in several countries with only the A2 variant of $\beta$-casein, which appeared not to have the disadvantages of A1 $\beta$-casein.

The second part of this review is a critique of the A1/A2 hypothesis. For both DM-I and CHD, the between-country correlation method is shown to be unreliable and negated by recalculation with more countries and by prospective studies in individuals. The animal experiments with diabetes-prone rodents that supported the hypothesis about diabetes were not confirmed by larger, better standardised multicentre experiments. The single animal experiment supporting an A1 $\beta$-casein and CHD link was small, short, in an unsuitable animal model and had other design weaknesses.

The A1/A2 milk hypothesis was ingenious. If the scientific evidence had worked out it would have required huge adjustments in the world's dairy industries. This review concludes, however, that there is no convincing or even probable evidence that the A1 $\beta$-casein of cow milk has any adverse effect in humans.

This review has been independent of examination of evidence related to A1 and A2 milk by the Australian and New Zealand food standard and food safety authorities, which have not published the evidence they have examined and the analysis of it. They stated in 2003 that no relationship has been established between A1 or A2 milk and diabetes, CHD or other diseases.

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## Build-up of the case

An ingenious hypothesis was developed by RB Elliott and CNS McLachlan and collaborators in the 1990s, that a protein in the milk of some cows—not others—is an important risk factor for type I diabetes (DM-I) and coronary heart disease (HD) (possibly also schizophrenia and autism). The implicated protein is the A1 form of $\beta$-casein, the second most abundant protein in cows milk: its commonest genetic variants are A1, A2 and B $\beta$-casein.

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### Type I diabetes mellitus

Elliott (1992) was struck by the very low incidence of DM-I among children in Polynesian islands like W. Samoa, compared with Polynesian children in Auckland. Prolonged breast feeding appears to be protective against DM-I. Cow's milk antibodies are found at higher levels in diabetic children than in controls. DM-I rates between countries correspond fairly well with cows milk intake.

The next step was animal experiments with a strain of mice genetically susceptible to diabetes, the non-obese diabetic (NOD) mouse. When fed for 250 days from weaning on diets containing A1 $\beta$-casein nearly half became diabetic but no diabetes occurred in the mice fed A2 $\beta$-casein.

Cows' milk $\beta$-casein contains 209 amino acids. The A1 and A2 variants differ only at position 67, which is histidine in A1 or proline in A2 milk. (Another variant B $\beta$-casein also has histidine at positive 67. It is less frequent than A1 or A2 in the milk of cows of European origin.) A bioactive

seven-amino-acid peptide, β-casomorphin-7 (BCM-7) can be released by digestion in the small intestine of A1 β-casein with pepsin, leucine aminopeptidase and elastase but the alternative proline at position 67 prevents a split at this site.

$Tyr^{60}$-$Pro^{61}$-$Phe^{62}$-$Pro^{63}$-$Gly^{64}$-$Pro^{65}$-$Ile^{66}$($His^{67}$)
β-casomorphin-7

BCM-7 has opioid and cytomodulatory properties (Meisel, 2001). Synthetic BCM-7 can inhibit responses of lymphocytes to stimulants *in vitro* (Elliott, 1992; Elliott *et al*, 1997). Elliott *et al* (1997) reported that NOD mice fed A1 β-casein did not develop diabetes if they were also given naloxone (the morphine antagonist). The antibody response to ovalbumin was prevented in NOD mice if they were also given injections of (synthetic) BCM-7; this prevention did not happen in Swiss mice. They suggested that appearance of diabetes in genetically susceptible NOD mice fed A1 β-casein—not those fed A2 β-casein—might be due to release from A1 β-casein of the bioactive peptide, BCM-7 which had a strong inhibitory effect on immune function.

A New Zealand patent, No. 295774 (1994) was registered in 1994 by the New Zealand Dairy Board and the NZ Child Health Research Foundation for *A Method of Selecting Non-Diabetogenic Milk or Milk Products* (New Zealand patent application 295774, 1994). 'This invention relates to a method for avoiding the triggering of Type 1 diabetes in humans by the ingestion of milk or milk products …' The A1 variant of β-casein does have diabetogenic activity in NOD mice, while the A2 variant and whey protein do not show diabetogenic activity.

A1 β-casein is a major variant of β-casein in the milk of the common dairy cows of north European origin: Friesian, Ayrshire, British Shorthorn, and Holstein. Predominantly A2 β-casein is found in the milk of Channel Island cows, Guernsey and Jersey, in Southern French breeds, Charolais and Limousin (Ng-Kwi-Hang & Grosclaude, 1992), and in the Zebu original cattle of Africa. For example, in British retail milks A1 ranged from 40 to 50% of the β-casein, A2 from 43 to 52% and B β-casein was 6 to 12% of the β-casein, but Jersey milk was an exception with much less A1 and more A2 β-casein (Hill JP, 2003, personal communication).

Fraser Scott (1990) in Ottawa produced graphs in which the incidence of DM-I could be seen to correlate between countries negatively with breast feeding prevalence at 3 months (17 countries) and positively with unfermented milk proteins, g/day (in 13 countries).

DM-I is rare in children of the Masai in E. Africa, a tribe with high consumption of milk from Zebu cows (Bos indicus). Elliott *et al* (1999) collected published data for diabetes incidence in children (0–14 y) in 10 developed countries and calculated the consumption of the total milk protein and of A1 and B β-casein. They used FAO data for national milk protein consumption, information and breed composition of the cows and their milk protein polymorphism. They found that total milk protein did not correlate significantly with DM-I but A1 β-casein did and correlation was even stronger with A1 and B β-casein (the latter also has histidine at position 67). Only a limited number of countries were included; they were countries where the incidence rates for DM-I were determined by similar methods and for which consumption of A1 and B β-casein could be reasonably estimated.

A second New Zealand patent application, No. 314285 (1998) '*Immune response diagnostic test*' was filed in 1998 by the NZ Dairy Board and NZ Child Health Research Foundation. This application stated …. 'Type 1 diabetes is induced by certain β-casein variants (most notably β-casein A1) and not by other β-casein variants (most notably β-casein A2) of milk ….'

> ''It is known that the A1 variant of β-casein induces a Type 1 diabetes immune response. It is believed on the basis of what is known in general about immune responses that the A1 variant may induce other immune responses of importance to the health of individuals. The present invention is not limited to determining the susceptibility of individuals to type 1 diabetes but includes diagnosis of other immune condition which might be caused by the active peptide.''

**Coronary heart disease**

Meanwhile CNS McLachlan, also in Auckland, New Zealand produced evidence for a correlation of mortality from CHD in 16 countries with national A1 β-casein consumption (g/day). McLachlan omitted A1 β-casein in cheese and β-casein type B. The numbers he calculated for national A1 β-casein consumption were not the same as in Elliott's correlations. He chose 5 y as the lag phase between food intake and CHD mortality. McLachlan's evidence was discussed within the dairy industry and in 1996 he applied for patents in New Zealand (McLachlan, 1996a) and with the WTO (McLachlan, 1996b), contending that consumption of a specific common variant of the milk protein β-casein (β-casein A1) promotes the development of heart disease in humans. His data were not published in the scientific literature until 2001 in Medical Hypotheses (McLachlan, 2001).

To add to this ecological data, Tailford *et al* (2003) reported a rabbit experiment in which rabbits killed after feeding for 6 weeks with 10% A1 β-casein showed larger areas of aortic fatty streaks than animals that received A2 β-casein. There were six rabbits per feeding group; areas showing fatty streaks were all small and serum cholesterols were higher in the A1 than the A2 β-casein group.

More extensive correlation calculations of A1 β-casein and other dietary variables against DM-I and CHD were published by Laugesen and Elliott (2003) in the New Zealand Medical Journal (Laugesen & Elliott, 2003). They concluded: 'Cow A1 β-casein per capita supply in milk and cream (A1/capita) was significantly and positively correlated with ischaemic heart disease (IHD) in 20 affluent countries five

years later over a 20 year period—providing an alternative hypotheses to explain the high IHD mortality rates in northern Europe compared to southern Europe'.

> ''For DM-I incidence at ages 0–14 years this study confirms Elliott's 1999 correlation on 10 countries for A1/capita, but not for B $\beta$-casein/capita. Surveys of A1 $\beta$-casein consumption in two-year-old Nordic children, and some casein animal feeding experiments, confirm the A1/capita and milk protein/capita correlations. They raise the possibility that intensive dairy cattle breeding may have emphasised a genetic variant in milk with adverse effects in humans. Further animal research and clinical trials would be needed to compare disease risks of A1-free versus 'ordinary' milk (Laugesen & Elliott, 2003).''

A company, A2 Corporation was set up with its head office in New Zealand and with international investors. The aim of the company is to sell milk containing A2 but not A1 $\beta$-casein. Those who want to avoid the unproven but credible risks associated with A1 milk but not A2 milk can choose to buy A2 milk. A method for identifying A1 and A2 has been developed and the company has the patent to test herds and produce pure A2 milk. A royalty is paid by the milk processor to the A2 company. So-called pure A2 milk also branded as Just A2 Milk where available is sold at a premium. In New Zealand, this milk has been shown to contain the A1 variant of $\beta$-casein and is thus not pure (New Zealand Commerce Commission media release 21 November 2003, see www.comcom.govt.nz).

A2 Corporation petitioned the Australian and New Zealand Food Authority to require a health warning on ordinary milk (containing A1 $\beta$-casein).

Individual dairy farmers in Australia, New Zealand and UK have been offering A2 milk to some supermarkets. A2 Corporation signed a deal with US corporation Idea-Sphere to sell A2 milk in US retail food outlets.

On the BBC News, 7 August 2003.

> People in the UK could soon get the chance to buy a type of milk which, it is claimed, could be safer for the heart than ordinary milk.
> Most of the milk sold in the UK contains particular proteins—called 'A1'—which some researchers have claimed could increase the risk of heart disease.
> Although this link has not been scientifically proven, producers in the UK are preparing to offer an alternative—a milk which does not contain the A1 proteins.
> The 'A2' milk would be marketed at approximately five pence a pint more than standard milk.
> It comes from herds which naturally produce milk with much lower levels of the A1 proteins.
> Many farmers in Australia and New Zealand have A2 herds, and the milk is popular among consumers there.
> In the UK, only a few herds in the Channel Island of Guernsey are A2 producers.
> Companies planning to market A2 milk say it is hard to satisfy demand.
> Farmer Rod Kent from Berkshire plans to own the first A2 herd in the country. His cows are being tested to make sure they are all A2 producers. He told the BBC: 'All milk is good for you, but if this is good for some particular small segment of the population let them have the choice'.

However, Professor Jeremy Pearson, from the British Heart Foundation, said that the advantage, if any, would probably only be slight. He said 'I think there would be far more effect on the incidence of heart disease from switching from full fat to semi skimmed rather than from switching from A1 to A2'.

## Critique of the hypothesis

### A1 milk and diabetes mellitus type 1 (DM-1)

Reasons why the between-country correlations are far from conclusive are as follows:

- Were the individuals who developed diabetes the ones who took the A1 $\beta$-casein? What human evidence we have suggests that any influence of milk (not just A1) on DM-I operates in infancy (Borch-Johnsen *et al*, 1984; Mayer *et al*, 1988) and this is usually consumed in the form of infant formulas. Only a small percentage of total milk consumption in any particular country is in infant formulas. National average consumption estimates for milk and products cannot serve as quantitative assessment of the A1 $\beta$-casein consumption from infant formulas. These formulas usually contain increased whey and reduced casein and the milk protein used for their manufacture does not always come from within the country where the formulas are used.
- Confounding cannot be excluded. To take an example, people in Finland, with a high rate of DM-I have a high frequency of HLA haplotypes that indicate susceptibility to diabetes (Reijonen *et al*, 1991). The island of Sardinia has the highest DM-I incidence in the Mediterranean region; emigration studies and HLA types show that this is, in the main, genetically determined (Muntoni & Muntoni, 1999).
- Breast feeding can in some communities be negatively associated with DM-I in case–control studies (Jones *et al*, 1998). There are socioeconomic, and hence environmental differences between breast-fed and formula-fed infants and breast fed are not drinking A1 milk.
- Nutritional scientists have experienced the unreliability of correlation studies of food intake and chronic disease. It was earlier claimed that sugar consumption correlated with CHD (Yudkin, 1964); that countries' fat consumption correlated with breast cancer (World Cancer Research Fund, 1997) and that their meat consumption correlated with colon cancer (Armstrong & Doll, 1975). Closer

human research has shown these associations to be spurious or uncertain (FAO/WHO Expert Consultation, 1998; Committee of Medical Aspects of Food & Nutrition Policy, 1998; Truswell, 2002).

- There are particular difficulties with the A1 $\beta$-casein-DM-I correlations. There is more uncertainty with national figures for A1 $\beta$-casein than for total milk casein and more uncertainties for these than for average milk consumption. Some important developed dairy countries were not included, for example, Netherlands, Ireland, nor were any of the emerging and developing countries.

For Beaglehole and Jackson (2003), who wrote the editorial to accompany Laugesen and Elliott's article (2003), 'the epidemiological literature has many examples of this ecological fallacy' (ie between-country correlations). Questions can also be raised about inclusion of only 20 'health-care affluent' countries out of a possible 36 likely to be in this category …. 'the authors appreciate that the ecological study is only a starting point for the generation of hypotheses. It would be prudent, however, to suggest other observational study designs before embarking on …. difficult, complex and expensive clinical trials …. Further animal studies alone will never be sufficient for public policy decisions'.

For Altman, author of a major textbook of statistics for medical research (Altman, 1991) 'interpretation of international correlations is particularly difficult because there are so many differences between countries …. We ought not to take any correlations as indicating a causal association without collateral evidence, however, 'reasonable' the hypothesis may be'.

For Willett (1990), author of '*Nutritional Epidemiology*', 'another serious limitation of international correlational studies is that they cannot be independently reproduced which is an important part of the scientific process. Although the dietary information can be improved and the analyses can be refined, the resulting data will not really be independent; the populations, their diet and the confounding variable are the same. Thus, it is not likely that many new insights will be obtained from further ecologic studies among countries …. On balance, ecologic studies have unquestionably been useful, but are not sufficient to provide conclusions regarding the relationship between dietary factors and disease and may sometimes be completely misleading'.

A striking example of the unreliability of between-country correlations is the absence of correlation of average national tobacco products consumption against ischaemic heart disease mortality in Laugesen and Elliott's (2003) paper. Smoking is a very well-established risk factor within countries but the variability of other risk factors for this multifactorial disease between countries obscures its effect when national averages are compared.

Two animal strains have been used to serve as models for the development of DM-I in humans. The genetic component is strong in BioBreeding (BB) rats and NOD mice, as it is in human DM-I (Powers, 2001). The percentage of a feeding group of these experimental animals that develop diabetes varies with the weaning diet used.

With the BB rat, Scott (1996) found that wheat and soy were particularly diabetogenic. Several researchers have reported that addition of skim milk or cows' milk proteins or casein to basal diets increased the diabetes incidence in BB rats or NOD mice (references in Paxson *et al*, 1997). Two possible milk proteins that might stimulate islet cell damage were shown not to increase incidence of diabetes in NOD mice: bovine serum albumin and bovine immunoglobulin G (Paxson *et al*, 1997).

It was clearly essential to confirm independently Elliott's experiments with NOD mice (Elliott *et al*, 1997). A large three-centre experiment was therefore set up with experienced researchers in Ottawa (at Health Canada), in London, UK (at St Bartholomew's Hospital Medical School) and at the University of Auckland (where Professor Elliott was again the senior investigator). The methodology was meticulous and standardised. Nine different diets were made up at New Zealand Dairy Research Institute and sent out 'blind' to the three animal centres (Beales *et al*, 2000). Outcome results are in Table 1.

The experiment in London, England had 35 NOD mice in each of the nine feeding groups. After 250 days, the highest rate of diabetes was in the control group (fed on wheat, corn, soy, alfalfa, oats, fish meal and cellulose—no milk), see table. In Ottawa, there were 30 BB rats per group, all fed on experimental diets for 150 days. Here too the (same) mixed cereal-based control diet gave the (significantly) highest diabetes incidence. There was little difference between A1 and A2 $\beta$-caseins and none were statistically significant. The two types of $\beta$-casein were fed at 10%, either with 'pregestimil' (casein hydrolysate plus starch, oil, etc) or with prosobee (infant formula with soy protein, etc).

The Auckland trial was abandoned half way through the experiment after an outbreak of Clostridium sp. resulted in death of many of the animals. Up to this time, the pattern of results in the NOD mice here was similar to that seen at the other two sites.

**Table 1** Percent of rodents that developed diabetes (Beales *et al*, 2000)

| | *PG* | *PG + A1* | *PG + A2* | *PS* | *PS + A1* | *PS + A2* | *Control mixed diet* |
|---|---|---|---|---|---|---|---|
| London NOD mice | 36% | 33% | 38% | 17% | 30% | 32% | 71% |
| Ottawa BB rats | 39% | 27% | 35% | 39% | 46% | 19% | 73% |

PG = 'Pregestimil', PS = 'Prosobee'.

The pancreases were examined histologically and for cytokines. There were no consistent differences between A1 and A2 casein groups.

The distinguished international panel of authors of this important paper (Beales *et al*, 2000) concluded: 'A previous result that A1 $\beta$-casein was more diabetogenic than A2 $\beta$-casein in NOD mice was not confirmed …. These findings show that it is not likely that diabetes could be prevented solely by removing or altering the cow's milk component of the diet' and they focus 'attention on other diabetes promoting foods, particularly wheat'.

Results are equivocal whether any other milk protein is diabetogenic in animal experiments. Adding bovine serum albumin or complete milk protein to a diet based on hydrolysed protein did not affect the incidence of diabetes in BB rats (Virtanen *et al*, 1991; Malkani *et al*, 1997). Similarly, addition of skim milk to a standard diet did not affect the diabetes incidence rate in NOD mice (Coleman *et al*, 1990; Virtanen *et al*, 1991).

Human case–control studies have reported feeding histories in infancy and childhood in cases of DM-I, compared with controls, matched for age, sex and social conditions. As this diabetes starts at different ages in childhood, the details of infant feeding usually have to rely on the mother's memory, often of 10 or 15 years before. Gerstein (1994) found 13 reported case–control studies in eight countries, reviewed them systematically and carried out meta-analysis on those with the best design. In those studies that minimised the potential for bias, the risk of DM-I was about 1.5-fold increased with a history of early cows' milk exposure or less than 3 months breast feeding. Norris and Scott (1996) did another meta-analysis with 17 studies found in the literature (most of them of course the same). As well as questioning the reliability of early infant feeding in retrospective assessment of exposure, they pointed out the potential bias from different response rates of the cases and controls. In the minority of cases with infant feeding records (rather than mother's recollection), the odds ratio for DM-I was only 1.13 for not breast-fed. They concluded that the increased risk of DM-I associated with any of the infant food exposures is small.

In another study, Norris *et al* (1996) screened 253 children from families with DM-I for three serum antibodies to pancreatic islet $\beta$-cells, that is, the presumptive stage before clinical diabetes. In all, 18 cases were found: their infant feeding was no different from the controls.

Virtanen *et al* (2000) collected 36 children who developed diabetes and 7 times as many controls, all matched with HLA typing for genetic susceptibility to DM-I. Again, the proportion of cases and control subjects, who had been breast-fed for at least 2 months did not differ. In this study, however, the cases were more likely to have consumed three or more glasses of milk per day in childhood. Virtanen *et al* (1991) had earlier reported from a smaller study that breast feeding was protective against DM-I.

Kimpimaki *et al* (2001), some of them the same researchers and also from Finland, screened and followed infants at genetic risk of DM-I for the appearance of islet cell antibodies. A total of 65 children who tested positive for four different auto antibodies (ICA, IAA, GADA and 1A-2A) were more likely than 390 controls to have had early exposure to cows milk or, put another way, only a short duration of breast feeding.

Appearance of serum autoantibodies associated with DM-I does not guarantee that diabetes will strike (Couzin, 2003). The ideal design to see whether breast feeding protects, or early milk formula increases the risk of DM-I would be a randomised controlled trial. A Trial to Reduce Insulin-dependent diabetes in the Genetically at Risk (TRIGR) has started, headed by Finnish researchers, with cases collected also in Europe, USA, Canada and Australia. Pregnant women are recruited who are DM-I or have a diabetic husband or child. Babies' cord blood is screened for higher risk genotypes. Qualifying infants receive either regular cows milk based formula or Nutramigen (a formula for babies with allergies in which the large proteins of cow's milk have been broken down) (Couzin, 2003).

All the countries where case–control studies have been conducted (Australia, Canada, Denmark, Finland, Germany, Norway, Sweden, UK, USA) have relatively high estimated A1 $\beta$-casein consumption. But even if there is a small risk of later DM-I for genetically prone infants fed cow's milk (formula) early in life, this may not be because of an effect of the milk but rather because of a decrease in protection of the immune system from a reduction or absence of mother's human milk. As the American nutritionists Goldberg *et al* (2002) conclude: 'The argument that milk consumption in childhood causes type 1 diabetes is equivocal at best'. That milk containing A1 $\beta$-casein causes DM-I is *a fortiori* even less likely. Proteins in soya and wheat seem to be more potent diabetogens than any in milk (Scott, 1995).

Whether and how milk $\beta$-casomorphin-7 is released in humans after drinking milk with its $\beta$-casein in A and/or B forms has never been clearly demonstrated (Hill *et al*, 2002). BCM-7 could not be demonstrated in human plasma after ingestion of cow's milk (Teschemacher *et al*, 1986). It was postulated by Elliott *et al* (1997) that BCM-7 acts on lymphocytes in the intestinal wall and in some way promotes an auto-immune reaction to insulin-producing $\beta$-cells resulting in their damage and so the required amount of insulin cannot then be secreted. However, Hartwig *et al* (1997) could not find that BCM-7 is released at all in the intestines in feeding experiments with lambs.

## A1 milk and CHD

The general reasons why between-country correlations cannot provide conclusions are set out in the corresponding section on A1 milk and diabetes (above).

A time lag of 5 y was assumed by McLachlan (2001) and by Laugesen and Elliott (2003) between A1 $\beta$-casein consumption and CHD mortality but this might be too short a time.

The effect of confounding is illustrated by lack of significant correlation between national tobacco consumption and CHD mortality in Laugesen and Elliott's (2003) paper. As smoking is a better established risk factor for CHD, this shows that we cannot rely on between country correlations to discover causes of CHD.

The use of average dietary consumption between countries against CHD incidence has been abandoned by all serious researchers since Yudkin's sucrose/CHD hypothesis was rejected in the early 1970s (Keys, 1971, 1973).

Focusing on details in the plot published by Laugesen and Elliot (2003) (which is the most complete and recent analysis from this group), there are whole countries that did not follow the average regression line. Austria and France were estimated to have similar $\beta$-casein consumption (0.93 g/day) but CHD mortalities of 88 and 33 per 100 000, respectively. Sweden, Australia, Iceland, Canada, Germany, Israel and the island of Jersey all had CHD mortalities between 70 and 80 per 100 000 but their A1 casein consumption ranged from nearly the highest in the world (2.8 g/day in Sweden) down to the lowest (0.3 g/day in Jersey) (Laugesen & Elliott, 2003).

While CHD mortalities in different countries have been closely watched since the Second World War, rates have come down in some countries—Finland, USA, Australia particularly—and gone up in other countries, particularly in eastern Europe. In Switzerland, there is reliable data on milk consumption and on health statistics. While milk protein consumption has been static at about 25 g/head/day, the CHD mortality moved down steadily from 160/100 000 in 1980 to 95/100 000 per year in the mid-1900s (Hill *et al*, 2003). Crawford *et al* (2003) have recently published the results of multiple computations, using CHD mortality data from WHO and food consumption data from FAO for 47 countries (from Argentina to Uruguay), for five dietary components (milk protein, alcohol, cheese, meat and coffee). Correlations were computed of each dietary component against CHD mortality for the 47 countries for every second year from 1969 to 1995. These correlations were computed for same year data and for successive time lags up to 30 y. The results are remarkable. Correlations with milk protein were around 0.7 until 1983. Since then they have come down to zero. The authors say that for most countries, the proportion of the A1 $\beta$-casein variant is in the range 30–55% of total $\beta$-casein, so that a correlation with CHD mortality should be observed if consumption of A1 $\beta$-casein is causative The different lag times made very little difference to the correlations between CHD mortality with milk protein consumption.

The other four dietary components tested all had positive but lower correlations; they have all declined since around 1983 and are now less than zero. Correlations with cheese were never very high, between $+0.2$ and $+0.3$; they are now $-0.3$.

Two possible mechanisms for an effect of milk protein on CHD have been proposed in the nutrition literature. One of these is a plasma cholesterol-raising effect of casein, when compared with soya protein, best seen in rabbits. In human experiments, however, when casein is exchanged for soya as the main dietary protein there have been little or no differences in plasma cholesterol (van Raaij *et al*, 1979, 1981, 1982; Grundy & Abrams, 1983). The bovine caseins used presumably included mixed A1/A2 $\beta$-caseins; the experiments quoted were conducted in the Netherlands and the USA. Sacks *et al* (1983) concluded: 'Casein, in amounts common to American diets, has not been shown to modify the plasma lipid levels of adults. Thus from the point of view of practical clinical nutrition non fat dairy products may be utilised in plasma lipid lowering diets'.

The idea of a cholesterol-lowering milk factor arose from observations on the Masai tribe in East Africa, some of whom consume large amounts of fermented milk but have low plasma cholesterols and seldom experience CHD. Some nutritionists reported that free-living subjects could drink large amounts of whole milk without raising their plasma cholesterol (Howard & Marks, 1977, 1979; Mann, 1977). In people who drink several litres of milk per day, the balance of the diet must be affected. With more ordinary intakes of milk (1–2 l/day) in studies under intake-controlled metabolic unit conditions, whole milk did raise plasma cholesterol somewhat and skimmed milk did not lower it (Hussi *et al*, 1981; Roberts *et al*, 1982; Howard & Marks, 1982). It is now generally accepted that there is no obvious cholesterol-lowering factor in cows' milk.

Neither a possible cholesterol-raising effect of casein nor a cholesterol-lowering effect of the aqueous phase of milk appear to be available mechanisms for any effect of A1 milk on CHD. No plausible mechanism has been put forward for a different influence on the pathogenesis of CHD between A1 and A2 milk.

Only one animal experiment has been published, which bears on the A1/A2 milk question (Tailford *et al*, 2003). The experiment was very short, for only six weeks; the diet groups were very small, only six per group, and the animal model, rabbits (whose normal diet is leaves) were fed on highly artificial diets, including 20% animal proteins. This is not a realistic model for human atherosclerosis, which develops over years and is different histologically from the small patches of early fatty streaks seen in the Tailford *et al* experiment. Some differences were reported in plasma cholesterols between rabbit feeding groups on this fairly extreme diet (the rabbits lost an average 5–6% body weight), but this cannot be taken to mean the same would happen in humans—human subjects plasma cholesterols have not been compared when eating A1 $\beta$-casein *vs* A2 $\beta$-casein. Measurement of the aortic fatty streaks was not made 'blind' to the diet group and the difference in areas between A1 and A2 were not significant in aortas of the animals also given cholesterol in their diets, or in the carotid arteries (Tailford *et al*, 2003).

In the accompanying editorial to the Tailford *et al* paper in *Atherosclerosis*, Mann and Skeaff (2003) explain that 'there are tremendous limitations to extrapolating these results to clinical effects in humans. … To even speculate that the findings should be extrapolated to public health measures

would seem to be irresponsible …. Far more convincing epidemiological, clinical and animal model evidence than exists for β-casein A2 suggested that high intakes of vitamin E … were associated with cardiovascular risk reduction. However, several large randomised controlled trials of vitamin E supplements have not been able to confirm evidence of benefit' (Mann & Skeaff, 2003).

In a large prospective epidemiological study, Ness *et al* (2002) reported results for coronary heart disease mortality and all causes mortality in a cohort of 5765 men recruited from work places in the west of Scotland and followed up for 25 years. At the original examination one of the questions asked was 'how many pints of milk do you usually drink each day?' The authors did not ask if it was reduced fat milk; such milks were little used by male workers in 1970–1973. All causes of mortality and deaths from CHD and from stroke showed inverse associations with milk consumption. It was possible that men who drank more milk had healthier lifestyles, leading to confounding, so the data were adjusted for age, smoking, blood pressure, cholesterol, body mass index, social class, education, car use, bronchitis and alcohol consumption. Still, the adjusted relative risk (RR) in the 2350 deaths from cardiovascular disease was 0.93 in those who drank a pint of milk a day and 0.64 in those who drank two pints (the trend was significant, $P=0.05$) (Ness *et al*, 2002).

Ness *et al* (2002) collected seven other prospective studies that have examined the association between milk consumption and coronary, cerebrovascular or all causes mortality. In none of them was milk consumption associated with more cardiovascular or total mortality. They are summarised in the following notes:

Snowdon *et al* (1984) reported a cohort of 25 000 Seventh Day Adventists. RR in men drinking two glasses of milk per day was 0.94 (significant) but for women it was 1.1 (not statistically significant).

In the Honolulu Heart Study (Abbott *et al*, 1990), in 3150 men (of Japanese ethnic group) those who drink 16 oz (474 ml) per day had half the risk of stroke of nonmilk drinkers.

In the Caerphilly cohort of 2818 men in South Wales (Elwood *et al*, 1991) the RR of CHD for those drinking one or more pints (0.568 l) per day, compared with those drinking none, was 0.13 (highly significant) (See note).

In the British Regional Heart Study (Shaper *et al*, 1991) after adjustment for smoking, socioeconomic position and other coronary risk factors, the RR for cardiovascular disease (fatal and nonfatal) in 7735 men was 0.88 in those who drank milk or had milk on their cereal (cf men who did not).

In the Basel study (Stahelin *et al*, 1992) of 2974 men working in the pharmaceutical industry, followed for 8 y, milk consumption and coronary atherosclerosis (at necropsy) showed an inverse correlation ($P=0.12$).

In over 10 000 British vegetarians (Mann *et al*, 1997) all causes mortality was lower in those who drank more than 1/2 pint of milk (285 ml) compared with those who drank less. CHD mortality, however, was not lower.

In over 34 000 middle-aged women in Iowa (Bostick *et al*, 1999) the adjusted RR for CHD mortality was 0.94 (not significant) in those in the top quartile consuming dairy products, excluding butter.

Thus, although much of the milk would have been full cream whole milk, with its plasma cholesterol-raising fatty acid pattern, eight prospective studies have not shown higher rates of CHD or cardiovascular mortality in people who said they drank more, rather than less milk at the start of the follow-up period.

Six of these prospective studies were in the UK and USA, countries where Laugesen and Elliott (2003) estimated the consumption of A1 β-casein is relatively high. The more milk subjects drank in these countries, the higher their intake of A1 β-casein. These prospective studies of individual intakes would seem to negate the more indirect McLachlan (2001) and Laugesen and Elliott (2003) correlations that showed A1 β-casein consumption of selected countries related to CHD mortality.

The World Health Organization's Expert Consultation (WHO/FAO Consultation, 2003) on diet, nutrition and chronic diseases list 23 dietary factors as related (or not related) to cardiovascular disease. Milk does not appear in the summary table or in the text. Since most milks in most developed countries contain substantial proportions of A1 β-casein, it follows that this particular variety of a major bovine milk protein is not generally regarded as a risk factor for cardiovascular disease.

## Conclusions

### Type I diabetes

The hypothesis that A1 (and not A2) β-casein may increase risk of DM-I in genetically susceptible children envisages release of the opioid peptide, BCM-7, which in some way affects the immune system, so that auto-antibodies against pancreatic β-cells, are more likely to be formed. However, release of BCM-7 has not yet been demonstrated in human subjects. As the only difference between A1 and A2 bovine casein is the amino acid at position 67, the presence and activity of BCM-7 seems central to any difference between them in biological effect.

Correlation of DM-I incidence between (a selected number of) countries and estimated national average A1 β-casein consumption is only suggestive evidence. This method has proved unreliable in the past. One problem is that the national A1 β-casein consumption might be different from infants' intake in formulas (which have increased whey, reduced casein and may originate outside the country of consumption). In Switzerland, DM-I has increased three-fold since 1990 but milk protein consumption has not changed (Crawford *et al*, 2003).

The largest, multicentre and best controlled animal experiments with diabetes-prone strains of mice and rats did not show more diabetes in those fed with A1 β-casein than those receiving the same amount of A2 β-casein.

It is possible that early feeding of cows milk (not otherwise specified) to infants genetically susceptible to DM-I might increase the risk of their developing diabetes, but the published case–control studies include nearly as many that showed no risk. In meta-analysis, the relative risk is equivocally little more than 1.0. Any effect may because of protection from breast feeding is missed and there is no evidence which component of milk could have been responsible for the effect, if any.

*There is, thus no convincing or probable evidence that the A1 β-casein in cows milk is a factor causing DM-I diabetes*.

## Coronary heart disease

Between-country correlations are known to be an unreliable method in searching for causes, for example, they show no positive correlation of tobacco consumption with CHD (Laugesen & Elliott, 2003). In Jersey, where estimated A1 β-casein consumption is minimal, the death rate from CHD is about the same as in Australia (with 41% A1 in its milk). Multiple recalculations by Crawford *et al* (2003) of the correlation coefficients for different foods, for different years and using a range of time lags and a larger set of 47 countries show that correlations of milk protein with CHD mortality have now dwindled to zero, perhaps along with recent changes in countries' coronary mortality.

I have seen only one short animal experiment (Tailford *et al*, 2003) comparing A1 and A2 β-caseins relevant to CHD, carried out in rabbits. It has several defects of design and was in an inappropriate animal model. No mechanism has been presented for any differential effect of cows milk β-casein types on the pathogenesis of CHD.

Meanwhile CHD mortality has declined considerably in countries like USA, Australia and Switzerland without reduction in milk and cheese protein consumption. The available human prospective epidemiological studies show no increased CHD in people who drink more milk.

*There is thus no convincing or probable evidence that the A1 β-casein in cows' milk is a factor causing CHD*.

The evidence relating autism and schizophrenia to A1 or A2 β-caseins in milk is more speculative and the evidence is more unsubstantial than that for DM-I and for CHD.

## Note

Since this paper was submitted, Elwood *et al* (2004) have published a later follow up of their cohort which confirms negative association of milk drinking with CHD and stroke (Elwood PC *et al* (2004) European Journal of Clinical Nutrition 58: 711–717).

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