

Heart Disease, Diabetes, Gut Immune Suppression and Epidemiology Studies

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Abstract

The consumption of cow's milk containing the β -casein variant A¹ has been linked with type 1 diabetes (insulin-dependent diabetes mellitus; IDDM) in both NOD mice and BB rats. Supporting this, epidemiological studies that include both inter- and intra-country data yield a strong association of this protein's presence in milk with the incidence of IDDM. A stronger association can be observed when correlating β -casein A¹ consumption with ischaemic heart disease (IHD) mortality, with a significant regression correlation coefficient ($r^2 = 0.86$). This suggests that the rate of β -casein A¹ consumption, excluding that contained in cheese, is a more accurate predictor of heart disease between and within countries than that reported for traditional risk factors. It is often assumed that the response rates of illness to dietary inputs are dose specific. Should this not be the case, as animal studies of both IHD and IDDM indicate, then positive, null or negative outcomes will be achieved depending on the approach of the sample to a genetically at-risk group, or to the general population. In diseases such as those noted above where immune dysfunction or gut immune suppression appear to play a major role, failure to compare immune response may skew the data analysis and hide causality. Thus, the failure to detect strong associations between consumption of specific dietary components and diseases in studies of individuals, as is the case with both IHD and IDDM in the population at large, may reflect a non-linear relationship between dietary components and disease.

Keywords: casein, casomorphin, IDDM, IHD, diabetes, heart disease, epidemiology, immune.

INTRODUCTION

Epidemiological studies of cardiovascular disease have failed to provide explanations for why previously designated 'risk factors', such as raised serum cholesterol, hypertension, obesity and per cent dietary energy from saturated fatty acids, differ in their apparent relative contribution to the illness. This disparity appears from one decade to another, and between different communities.

The between-countries prediction of the rates of heart disease based on the measured risk factors can be described as mediocre at best [1]. Furthermore, Keys' fat relationship was not observed in within-community studies [2]. The summary provided by Table 1 shows that those who developed heart disease were found to have the same fat consumption as those who did not. Rose [3] suggested that in populations where the majority have a high fat intake, factors other than fat become important in determining why some individuals develop ischaemic heart disease (IHD) and others do not.

The results of the World Health Organization (WHO) Monitoring in Cardiovascular Diseases (MONICA) survey of the classically accepted risk factors identified with cardio-

TABLE 1. Intake of total fat and saturated fatty acids vs. ischaemic heart disease (IHD) in major prospective studies. Adapted from [3]

	Total fat (% total energy)			Saturates (% total energy)		
	No incidence IHD	Incidence IHD	Difference	No incidence IHD	Incidence IHD	Difference
Framington	38.8	40.0	+ 1.2	14.9	14.8	- 0.1
Puerto Rico (urban)	36.6	37.7	+ 1.1	13.5	13.3	- 0.2
Puerto Rico (rural)	32.2	32.0	- 0.2	12.6	14.0	+ 1.4
Honolulu	33.3	35.2	+ 1.9	12.3	13.0	+ 0.7
London	40.5	40.0	- 0.5	—	—	—
Zutphen	41.7	41.8	+ 0.1	17.6	17.7	+ 0.1
Ireland/Boston	38.5	39.4	+ 0.9	16.9	17.4	+ 0.5
Caerphilly	40.1	40.9	+ 0.8	29.5*	29.7*	+ 0.2

* Animal fat.

vascular disease, and their changes with time, have recently been reported [4]. In a survey of 39 centres in 26 countries, the conventional risk factors identified in the 1950s were found to be even less relevant. In males, the changes in the combined risk factors were found to account for about 40% of event rates, while in females they accounted for only 15%. These changes did not reflect the historical rise and fall of disease mortality in any community studied.

More disconcerting is the MONICA Toulouse/Belfast study [5]. Overall risk factors assessed by two multiple logistic function scoring systems were identical. There was no significant difference in macronutrient intake and no appreciable difference in total fat intake, although saturated fat consumption was higher in Belfast while cholesterol and polyunsaturated fat consumption was greater in Toulouse. Heart disease incidence and mortality were found to be three to four times as common in Belfast as in Toulouse.

Notwithstanding these findings, recent studies of the cholesterol-lowering group of drugs, the statins, found an approximately 30% reduction in deaths in takers of the drugs after a first cardiac event.

Heart disease risk factors may be crudely classified as inputs and outcomes; most involve immune suppression/compromisation.

In some instances, the risks classified as outputs will be part of a feedback mechanism as they become inputs. What is not clear is how the immune responses associated with the risk factors affect both general and gut immune function.

MILK CONSUMPTION AND IHD

Although inter-country correlations and regional analyses have thrown up strong links [6] between consumption of milk protein and heart disease, studies of individuals show contradictory results [7, 8] or no association, as recently reported in the west of Scotland study [9]. In the latter case, a prior report [10] noted that one of the shortcomings of the study was the omission of adequate dietary data.

However, there is a large body of literature on casein consumption and heart disease in animals [11–13]. Casein has been recorded as causing heart disease, or hypercholesterolaemia, in rabbits [14–20], rats, pigs [21] and monkeys [22,23]. The preceding references represent a small fraction of the numerous animal casein publications devoted to atherogenesis. Dietary studies of pigs also showed that they developed hyperhomocysteinaemia when fed a diet containing casein [24]. Hyperhomocysteinaemia is a significant risk factor for IHD in humans.

Apolipoprotein-E-deficient mice are currently regarded as one of the more appropriate models of human atherosclerosis because they develop severe hypercholesterolaemia and atherosclerotic lesions that are similar in distribution and appearance to those observed in humans [25, 26]. They are found to develop significantly more atherosclerosis lesions when fed casein rather than soy protein isolate, with or without the presence of added cholesterol in the diet [27].

The presence of casein in the diet of rats has been shown to affect the activity of hepatic lipoprotein receptors that regulate very low-density lipoproteins (VLDL) and/or β -VLDL uptake [28]. This phenomenon is not seen in soy-fed rats [29, 30]. Whey protein was found to produce lower liver cholesterol concentrations in rats than casein [31].

Ageing studies in rats [32, 33] and mice [34] have also shown that casein as the primary source of dietary protein leads to a significant reduction in life expectancy when compared with soy protein, or whey.

MILK CONSUMPTION AND DIABETES

The contribution that cow's milk may make to type 1 diabetes is still the subject of ongoing research. Most of the breast feeding studies that show a link between milk intake and

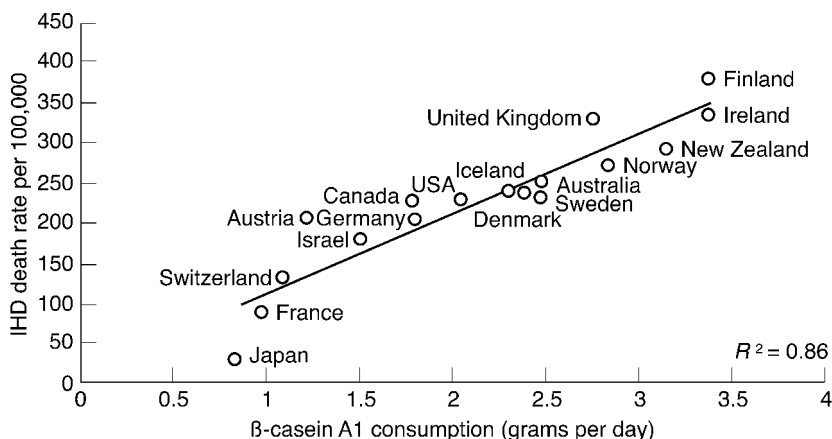


FIG. 1. Relationship between β -casein A¹ (excluding cheese) consumption and incidence of ischaemic heart disease mortality in males aged 30–39 years in 1985.

diabetes are retrospective and are generally based on volunteers from the whole population rather than children with high genetic risk. When high-risk children are analysed, the inverse association between breast feeding and diabetes becomes significant [35, 36]. In the case of milk consumption, the risk of diabetes among the siblings of children with diabetes was 5.37 times greater among high consumers than low consumers [37]. The consumption of cow's milk, excluding cheese consumption, has been correlated with insulin-dependent diabetes mellitus (IDDM) in Italy [38].

β -CASEIN STUDIES

IHD

McLachlan [39] has found that the consumption of β -casein A¹, excluding cheese, correlates with IHD mortality between countries, and within countries, where herd distribution and breed genotype have been recorded. The correlation coefficient is higher than any other risk factor associated with heart disease ($r^2 = 0.86$) (Fig. 1).

Low-density lipoprotein (LDL) oxidation is considered to be a primary step in the development of atherosclerosis in man [40]. Analysis of protein oxidation products isolated from atherosclerotic lesions implicates the tyrosyl radical, reactive nitrogen species, and hypochlorous acid in LDL oxidation [41]. Torreilles and Guerin [42] found that peptides from bovine casein hydrolysates could promote peroxidase-dependent oxidation of human LDLs. The reaction required casein-derived peptides with tyrosyl end residues. Casomorphin-7 is a potential source of a tyrosyl radical. It is produced from β -casein A¹ and β -casein B but not β -casein A² [43]. Thus, the generation of casomorphin-7 may be the pathogenic link between the ingestion of milk containing β -casein A¹ and the development of vascular disease, which in turn leads to heart disease.

IDDM

In 1999, Elliott *et al.* [44] published an inter-country correlation showing a strong association between β -casein A¹ and β -casein (A¹ + B) consumption and the development of IDDM. McLachlan [39] has since published a study showing a similar correlation over a wider range of countries (Fig. 2). Elliott *et al.* [44] extended this epidemiological link with

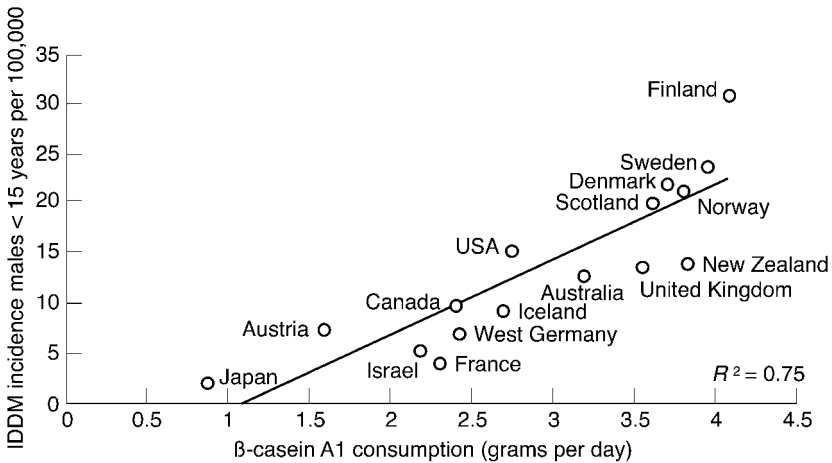


FIG. 2. Relationship between β -casein A¹ consumption and incidence of insulin-dependent diabetes mellitus in males < 15 years of age.

animal trials that served to identify β -casomorphin-7 as the putative diabetogenic agent. It is of note that two international patents have been filed in relation to these studies [45, 46].

β -casein levels have also been suggested to explain the difference in diabetes levels found in Iceland and Scandinavia [47]. Raised levels of β -casein antibodies in diabetic children have been reported in several studies [48, 49]. In most of these studies, raised levels of antibodies against other milk proteins were also recorded.

In contrast with IHD, the link between diabetes and the ingestion of β -casein A¹ appears more likely to be mediated by immune responses, although the possible role of casomorphin-7, identified [44] as a direct generator of free radicals, cannot be excluded.

DIETARY PROTEIN STUDIES

IHD

A negative dose-response effect of dietary protein content in experimental studies of heart disease has been observed in animals and birds [50, 51]. In pigs, 5% protein diets were found to produce more arterial lesions than 20% protein diets, both diets containing a common amount of cholesterol per animal body weight [53]. This effect has also been observed in rabbits, monkeys, dogs and pigs [53] and in rats [54].

IDDM

Studies of casein consumption and the incidence of diabetes in NOD mice measured by Elliott and Bibby [55] and on diabetes-prone BB rats by Scott [56] showed that rates declined significantly as the percentage casein in the diet increased. This led Scott [57] to conclude that the presence of casein in the diet of his animals was 'protective' against diabetes.

β -casomorphin fragments have also been shown to have a non-linear effect on the rate of insulin production from rat islets, depending on their concentration and the level of glucose present [58]. At low glucose levels they cause insulin stimulation, while at high levels they cause inhibition (Fig. 3).

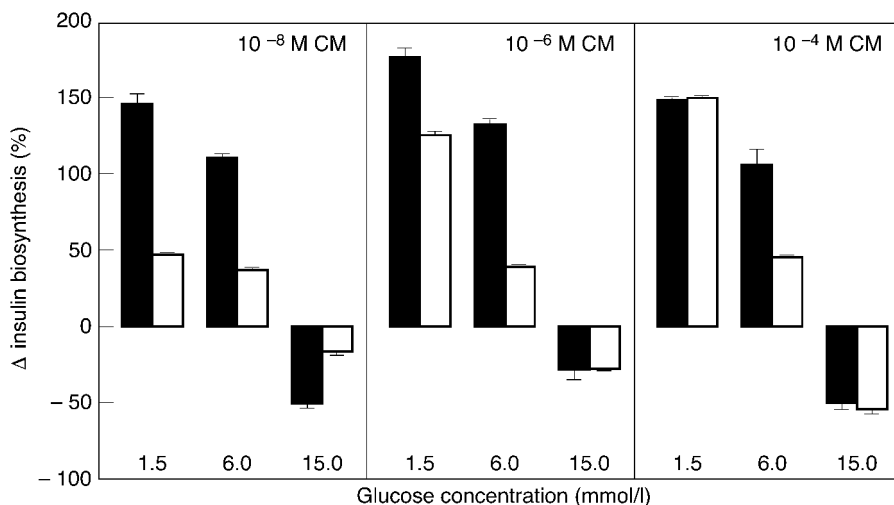


FIG. 3. Influence of β -casomorphins on insulin biosynthesis in isolated islets of Langerhans at various glucose concentrations. Adapted from [58]. Solid bars represent β -casomorphin (4), clear bars represent β -casomorphin (3)-N-Pyr. All values significant to $p < 0.01$.

EPIDEMIOLOGICAL CONCERNS

Kannel [59] has written that epidemiologically demonstrated associations are more likely to be causal if among other things they precede the disease and are strong and dose related and are consistent. Dietary studies on diseases where the strength, or depth, of the mediating factor cannot be measured, such as those involving gut immune compromise, may provide few useful outcomes. They may merely reflect gut permeability, being a function of the average gut immune state. The outcome is going to be dependent on the net overall immune state of the participants. On average, in a well-selected group, there should be a null result.

However, if one looks at people of a 'common' or dominating immune disposition, one should see an outcome. This appears to be the case in diabetes. Some diabetes studies show a link between milk consumption and diabetes risk, others do not. However, in the Finnish study of the siblings of children with diabetes, presumably a group with moderately similar immune qualities, the link was strong and positive with the risk of diabetes being 5.4 times for milk drinkers compared with that in light consumers [47].

There are similar relationships with diet in studies of heart disease. Studies on healthy people show little or no apparent response to milk consumption. However, in the Sippy diet study where milk was fed as a major component of the diet to patients with stomach ulcers, the death rates of those on the high milk content diet were two to six times higher than those of patients with a stomach ulcer but not on the diet [60] (Fig. 4).

Epidemiological studies examining food consumption and illness in individuals generally assume a dose-response relationship. That is, if a nutrient is illness causing then it is frequently concluded that there is a direct relationship between the amount consumed and the development of the illness. Such an assumption also does not appear to be the case in animal studies of IDDM, or of IHD.

THERMODYNAMIC CONSIDERATIONS INVOLVING PROTEIN EQUILIBRIA

Annand (1971) observed that following the introduction of Holder pasteurization of milk, deaths from heart disease doubled in 3 years in the various communities he examined [52].

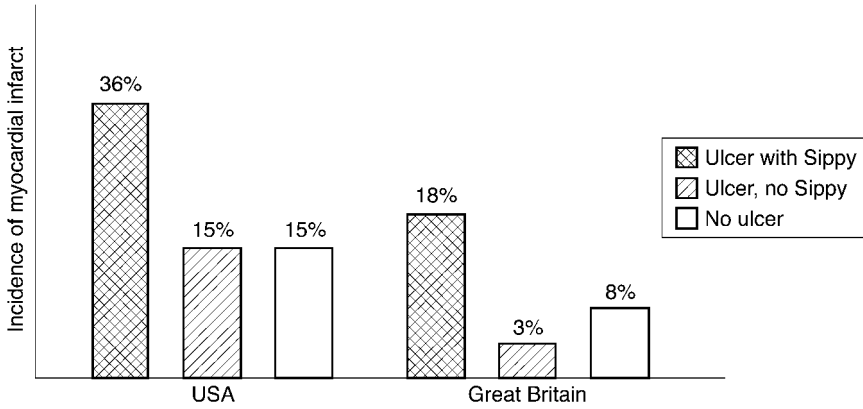


FIG. 4. Incidence of myocardial infarct among patients on Sippy or other high milk diets compared with controls matched for age, sex, race, hospital and year of death. Note that $n \geq 90$ for all groups. Adapted from [60].

Holder pasteurization involves heating milk to 63°C for 30 min. It is possible that the higher death rates recorded in the UK than in the USA in the Sippy diet experiments may reflect the fact that Holder pasteurization was still being used in some UK communities at the time.

Medical studies seldom treat nutrient consumption as a dynamic condition. The group of chemicals that makes up milk is involved in a number of complex thermodynamic equilibria, including those impacting on casein, which is present in whole milk as a micelle. During the process of digestion, the presentation of the protein mixture to the gut, and the place or time that a complex such as a micelle is being attacked, should reflect the thermodynamic condition of the associated species at the initiation and the first stages of digestion. Some effects are recognized, such as those involving protein denaturation, as is the case with the heating of the immunoglobulins.

In the case of milk, however, the thermodynamic relationship between monomeric caseins and the casein micelle may have been ignored. Theoretically, it would appear unlikely that casein in its micellar form would represent an immune threat to the gut. By contrast, transmission of monomeric β -casein, or digested fragments thereof, across the gastrointestinal mucosa may do. In solution, monomeric β -casein has an ionic structure similar to a detergent [61]. Studies of heated reconstituted milk show that, at $\text{pH} \geq 6.7$, monomeric β -casein levels peak at around 70°C, and will be between 10 and 15 wt % compared with ~ 5 wt % in unheated reconstituted milk, depending on the pH [62]. Comparative data for fresh milk have not been published. These effects provide a potential explanation for the Annand pasteurization observations. The fall in coronary heart disease deaths, which took place after the introduction of short contact time high temperature

TABLE 2. Heart disease risk factors divided into inputs and outcomes [4–6]

Inputs	Outputs
Smoking	Cardiovascular fitness
Saturated fat consumption	Obesity
Arterial infections	Elevated homocysteine
Diabetes	Raised serum proteins such as very low-density lipoproteins
	Insulin resistance

TABLE 3. Dose-response relationship in diabetes-prone experimental animals

% casein component added to diet	% of animals that develop diabetes	Animal type	Study [ref.]
2	19	NOD mice	Elliot <i>et al.</i> (1999) [55]
10	14	NOD mice	Elliot <i>et al.</i> (1999) [55]
20	10	NOD mice	Elliot <i>et al.</i> (1999) [55]
10	25	BB rats	Scott (1988) [56]
20	9	BB rats	Scott (1988) [56]
40	7	BB rats	Scott (1988) [56]

pasteurization, may well reflect a change in the level of monomeric β -casein present in the milk.

The implications that this consideration presents to epidemiology studies are notable. In the case of milk protein consumption, a simple measurement of intake in epidemiological studies may not measure the state of the thermodynamic equilibrium between monomer and micelle, so that data could be out, in this case, by a factor of two for the same milk intake, if monomer is the active ingredient.

CONCLUSION

Conventional epidemiology studies may be inappropriate as tools for evaluating dietary influences on human health in cases where a non-linear dose response to insult is believed to be present. Animal studies centring around the development of IHD and IDDM following dietary protein consumption appear to illustrate such a non-linear dose response. Furthermore, although such studies implicate β -casein A¹ as a highly significant risk factor for both IHD and IDDM, *in vitro* studies suggest that the putative causative agent derived from digestion products of the protein also exhibits a non-linear dose response.

Should these observations of non-linear illness responses to substances such as casein, proteins, and casomorphin fragments be confirmed in humans, epidemiology as a science needs to carefully reconsider how it evaluates associated human studies. Such considerations as the involvement of people sharing common immune or gut immune function and the processing of nutrient macromolecules may need to be taken into account to establish causality.

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