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Ischaemic heart disease, Type 1 diabetes, and cow milk A1 b-casein

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Abstract

Aim To test the correlation of per capita A1 ß-casein (A1/capita) and milk protein with: 1) ischaemic heart disease (IHD) mortality; 2) Type 1 (insulin-dependent) diabetes mellitus (DM-1) incidence.

Methods A1/capita was estimated as the product of per capita cow milk and cream supply and its A1 ß-casein content (A1/ ß) (calculated from herd tests and breed distribution, or from tests of commercial milk), then tested for correlation with: 1) IHD five years later in 1980, 1985, 1990 and 1995, in 20 countries which spent at least US $1000 (purchasing power parities) per capita in 1995 on healthcare; 2) DM-1 at age 0–14 years in 1990–4 (51 were surveyed by WHO DiaMond Project; 19 had A1 data). For comparison, we also correlated 77 food, and 110 nutritive supply FAO (Food and Agriculture Organization)-based measures, against IHD and DM-1.

Results For IHD, cow milk proteins (A1/capita, r = 0.76 , p <0.001; A1/capita including cheese, r = 0.66; milk protein r = 0.60, p = 0.005) had stronger positive correlations with IHD five years later, than fat supply variables, such as the atherogenic index (r = 0.50), and myristic, the 14-carbon saturated fat (r = 0.48, p <0.05). The Hegsted scores for estimating serum cholesterol (r = 0.42); saturated fat (r = 0.37); and total dairy fat (r = 0.31) were not significant for IHD in 1995. Across the 20 countries, a 1% change in A1/capita in 1990 was associated with a 0.57% change in IHD in 1995. A1/capita correlations were stronger for male than female mortality. On multiple regression of A1/capita and other food supply variables in 1990, only A1/capita was significantly correlated with IHD in 1995.

DM-1 was correlated with supply of: A1/capita in milk and cream (r = 0.92, p <0.00001); milk and cream protein excluding cheese (r = 0.68, p <0.0001); and with A1/ß in milk and cream (r = 0.47, p <0.05). Correlations were not significant for A2, B or C variants of milk β-casein. DM-1 incidence at 0–4, 5–9 and 10–14 years was equally correlated (r = 0.80, 0.81, 0.81 respectively) with milk protein supply. A 1% change in A1/capita was associated with a 1.3% change in DM-1 in the same direction.

Conclusions Cow A1 ß-casein per capita supply in milk and cream (A1/capita) was significantly and positively correlated with IHD in 20 affluent countries five years later over a 20-year period – providing an alternative hypothesis to explain the high IHD mortality rates in northern compared to southern Europe.

For DM-1, this study confirms Elliott's 1999 correlation on 10 countries for A1/capita,[1] but not for B ß-casein/capita. Surveys of A1 β-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.

In 1968, across 43 countries, Seely found that per capita milk supply (excluding butter) was highly correlated with IHD mortality rates 1 to 4 years later, and more strongly than animal fats or butter.[2] In 1968 also, came the first dairy science review of the genetic differences in milk proteins between individual cattle and breeds.[3] In North Europe, A1 was the predominant form of β-casein in cow milk (A1/β = 0.46–0.71) among the traditional black-and-white or red-and-white cow breeds (Red Danish, Holstein-Friesian, Ayrshire).[4] Artificial breeding during the 1970s and 1980s made use of American Holstein bulls (typically A1/β = 0.4), often replacing indigenous cow breeds, and reducing A1 levels in North European milk. In central and southern Europe, the A1/β fraction was lower due to the dominance of Jersey, Simmental or Swiss Brown cattle (A1/β mostly <0.25),[4] and virtually nonexistent in Guernsey cattle (A1/β = 0.01).[5] Except for milk from the island of Guernsey, commercially-sold European-breed cow milk is an A1 β-casein and A2 β-casein mix.

Ischaemic heart disease

Alerted by Elliott to the inter-country correlation between Type 1 diabetes and A1/capita in cow milk,[1] McLachlan patented a method to commercialise a possible relationship between A1/capita and IHD. In 2001, McLachlan published a 17-country ecological study, demonstrating a high correlation between A1/capita in the food supply circa 1980 and IHD mortality in 1985 and 1990.[6]

Fonterra Research Centre (FRC, formerly the Dairy Research Institute) scientists responded, noting that correlations found in past years between milk protein consumption and IHD across 40 countries (some high income, some low), were no longer found in the 1990s; observing any past correlation appeared to have been "serendipitous"; and that the evidence was not sufficient to warrant a change to A1-free milk.[7]

In examining these competing claims, we have put aside the question of biological mechanisms which these authors have touched on, and confined this study to the correlations. This study of IHD is limited to healthcare-affluent countries, to reduce inter-country IHD mortality differences due to disparities in the availability of coronary care. For comparison, we also examine the correlation of other food and nutritional supply variables with IHD.

Diabetes Type 1

Diabetes Type 1 (DM-1) incidence has been increasing globally at 3% per annum.[8] Its incidence varies by over 300-fold across 51 countries.[9] Although knowledge of genetic predisposition has increased, the nature of the precipitating environmental factors remains elusive. Across 12 countries, milk protein per capita and DM-1 rates were highly correlated.[10] Even when Finnish children of the same genetic susceptibility to DM-1 were compared, those consuming more than three glasses of milk daily remained at higher risk of DM-1 than those with a lower milk intake.[11]

No one could explain why Iceland, with high milk consumption, had a lower DM-1 incidence rate than the other genetically-related Nordic countries. Genetically-

predisposed non-obese diabetic (NOD) mice developed DM-1 when fed milk from the European Bos taurus cow, but not if fed milk from the Indian Bos indicis Zebu. Fed European cow milk casein, they developed DM-1, but not if fed cow milk whey (the other main protein fraction in milk) or soya protein. When fed the A1 variant of β-casein, the mice developed DM-1, but not when fed A2 or (fully) hydrolysed A1 β-casein. ($p = 0.002$) Also, A1 β-casein had no effect when given with naloxone, a morphine antagonist that opposes the opioid effect of β-casomorphin-7.[12] β-casomorphin-7 is a peptide formed by partial hydrolysis of A1, B or C β-casein only, the cleavage made possible by a histidine rather than a proline amino-acid at position 67 in these caseins. Milk-free cereal induced DM-1 in genetically-predisposed BB (BioBreeding Laboratories, Ottawa) rats[13] and in NOD mice also. Whole casein without cereal also induced DM-1 in NOD mice; whereas A1/β in BB rats had only a small effect, suggesting that even if milk were a factor in DM-1, some DM-1 would remain due to cereals in the diet.[14]

In a 10-country study in 1999, Elliott found that DM-1 rates were significantly correlated with A1/capita and particularly with the combined A1 and B variants (of β-casein) per capita.[1] In revisiting this study, we include nine more countries, and adjust for milk imports and their source, for the protein yield of each breed, and for the proportion of milk from other animals. We also estimate the A1/capita in the milk and cheese supply separately.

Methods

Countries selected These included all 22 countries (Tables 1 and 3) for which published A1/β cow milk data was obtainable, after: 1) excluding the Netherlands as simultaneous high imports and exports of milk precluded reliable determination of the origin of milk consumed (imports 65%; exports 85% of domestic usage in 1995); 2) for IHD only, excluding Hungary and Venezuela, as their total health expenditure was less than US $1000 per capita in 1995 (based on purchasing power parities),[15] leaving for study 20 "healthcare-affluent" countries (17 of which were OECD member countries, out of 22 healthcare-affluent countries and 29, in total, in the OECD in 1995[15]) 3) for DM-1 only, excluding countries not surveyed by WHO DiaMond Project or EURODIAB ACE (Ireland, Jersey and Guernsey), leaving 19 for study.

Milk and cream supply Milk and cream supply per capita was calculated from the nutritional statistical databases at the FAO (Food and Agricultural Organization) web site,[16] as milk protein per capita in grams per day. This was calculated as [3.3% by weight of 'milk excluding butter' + 2.7% of cream, minus 25% of cheese]. Milk included fresh milk products – yoghurts, cream, whole and skim milk, and milk powder – but excluded cheese and butter. We subtracted goat and sheep milk production (FAO data, Italy 8%, Israel 2%, Hungary 2%). Where imported milk comprised 20% or more of domestic milk usage (Germany, Italy, Japan),[17] we adjusted for the imported tonnage and the A1/β of milk in the main supplying countries, and similarly for cheese imports.

Nutritional data FAO food supply data[16] (unavailable for the Channel Islands) were converted to nutritional measures using British food composition tables, from Health New Zealand's food and nutrition database, listing values for 77 foods and 110 nutritional measures of national food supplies.[18]

Cow breed distributions Iceland, Norway, Jersey, Guernsey traditionally, and Israel and Japan in recent decades, were virtually one-breed countries. For other countries, we calculated the breed distribution from governmental animal census data,[19] from industry,[20,21] and otherwise from national breeding programme data.[22]

A1/b **and other** b**-casein fractions** These were estimated by breed from the dairy science literature held by the Fonterra Research Centre (FRC) for 18 countries. In addition, factory or retail milk was tested from 11 Table 1 countries during 1998–2001. Genotype estimates were based on published A1/β herd tests, for Austria,[23] Canada,[24] France,[25] Germany,[26] Hungary,[27] Japan,[28] New Zealand,[29] Nordic countries,[30] Switzerland,[31] the United Kingdom,[3] and the United States.[32] For the new Israeli Holstein breed,[33] we assumed US Holstein averaged A1/ß values from US herd test reports from 1968,[3] 1971,[34] and 1989.[32] For Italy, Professor F Addeo supplied data on 23 breeds, (personal communication, June 2000). The A1/ß fractions for breeds in Ireland and the Channel Islands were those of the same breeds tested in mainland Britain. In Iceland, tests were of herds[6] and of bulk milk samples.[30,35] In the absence of recent breed estimates or market share data, we used FRC test results on milk or milk powder from Australia (average of two samples) and Venezuela (average of 17 brands). During 1998–2001, milk test results were obtained from an additional nine countries: 1) from the Nordic countries;[35] and 2) from Canada, Italy, New Zealand, and the UK, tested by FRC. For DM-1, 1990 and 1995 A1/β data were averaged to represent the 1990–4 fraction in the national milk supply.

A1/capita in 1990 (IHD) and in 1990–4 (DM-1) was estimated thus:

A1/capita = (cow%) x (milk protein supply/capita) x (b**-casein/cow milk protein) x (national A1/**b**)**

Cow% is total milk production percentage minus the percentage from sheep and goat milk. Milk protein supply/capita is defined above. β-casein as a fraction of cow milk protein = 0.284.[36] National A1/β = sum of the percentage contribution of each breed, weighted for its percentage of the national dairy cow population,[19–22] the protein content of its milk,[22] and average milk yield of its cows.[22]

Mortality data WHO[37] and its website (www.who.int) supplied total cardiovascular disease (CVD), IHD, and cerebrovascular (stroke) mortality data for 18 countries, and Channel Islands data were supplied by their Departments of Health. The annual mortality rate per 100 000 population in the age group 35–64 years was standardised by averaging of the rates for the component three ten-year age groups, and then averaging male and female rates. A lag of five years was allowed from food supply to IHD mortality.[38]

DM-1 incidence data This was provided by the 1990–94 WHO DiaMond Project,[10] except for Iceland and Switzerland which were surveyed by the EURODIAB ACE Study group (Table 1).[39] Within each country, regional incidence results were averaged. Some were national surveys (Table 1). Rates for age 0–14 years were standardised by averaging six rates: 0–4, 5–9 and 10–14 year age groups by gender.

Food variables Over 75 food and over 100 nutritional food supply variables for 1990 for all Table 1 countries (except Venezuela due to lack of data) were obtained from FAO[16] and from Health New Zealand's food and nutrition database[18] and tested for correlation against DM-1 in 1990–4.

Table 1. Supply variables in 1990 and income and ischaemic heart disease rate in 1995, 20 countries, ranked on mortality

Country	GDP / capita	Tobacco products minus cigars	Alcohol	Wine	Athero-genic index	Myrist-ic fat C14.0	Hegsted score	Satur-ated fat	Milk & cream protein /capita	A1/ß in milk & cream	A1/ capita	IHD 35–64 years*
Year	1995 current US$PPP	1990 g/adult/ year	1990 g/day	1990 g/ day	1990	1990 % E	1990 g/100dl	1990 % E	1990 g/day	1990 fraction	1990 g/day	1995 mortality per 100 000
Japan	22 644	3212	12.9	3.2	0.36	0.64	139	7.2	4.9	0.52	0.73	24.7
France	20 197	2204	22.9	183.6	0.71	1.78	219	16.2	9.3	0.35	0.93	32.8
Guernsey	21 013	2241	24.8	58.2	NA	NA	NA	NA	11.0‡	0.01	0.03	43.5
Italy	20 119	1907	17.6	167.6	0.55	1.11	221	12.7	8.7	0.48	1.19	50.5
Switzerland	25 661	3733	17.9	119.5	0.73	1.75	215	14.1	20.0	0.21	1.20	51.1
Jersey	22 855	NA	27.0†	NA	NA	NA	NA	NA	11.5	0.09	0.29	69.9
Israel	11 748§	2335	2.3	6.6	0.51	1.20	161	11.3	7.8	0.42	0.91	70.8
Australia	21 845	2733	16.5	50.6	0.78	1.72	217	14.8	17.1	0.41	2.00	72.3
Iceland	22 250	2255	8.0	14.9	0.97	2.13	211	16.6	19.1	0.34	1.82	72.5
Sweden	19 952	1711	13.0	35.0	0.78	1.94	218	14.4	21.6	0.46	2.82	78.3
Denmark	22 974	2333	17.6	59.8	0.74	1.72	234	15.9	11.4	0.51	1.65	84.9
Germany	21 404	2535	24.3	73.3	0.72	1.74	227	16.8	8.6	0.43	1.06	81.2
Canada	22 901	2574	10.3	17.8	0.57	1.61	201	13.5	13.0	0.52	1.90	78.6
Norway	23 306	1871	7.8	18.8	0.72	1.81	200	13.8	15.2	0.46	1.99	91.1
Austria	21 454	2301	22.4	94.2	0.65	1.61	227	15.9	15.5	0.21	0.92	88.2
USA	27 895	2720	14.6	20.9	0.57	1.25	198	12.8	14.5	0.39	1.60	99.8
Finland	18 856	1933	16.4	11.9	0.94	2.02	211	14.2	22.8	0.48	3.11	113.0
NZ	17 051	1971	15.8	32.1	0.93	2.17	251	17.3	17.1	0.50	2.42	116.0
UK	18 630	2233	15.0	32.0	0.62	1.59	202	13.4	15.2	0.53	2.31	117.4
Ireland	18 117	2279	16.2	11.7	0.85	1.76	225	14.5	22.9	0.59	3.84	131.1
MEAN:	**21 241**	**2373**	**16.2**	**53.0**	**0.71**	**1.64**	**210**	**14.2**	**13.4**	**0.40**	**1.63**	**78.4**

* age standardised by averaging of the three ten-year age groups, with male and female rates averaged; †average, 1994–8; ‡1995; §not adjusted for purchasing power parities (PPP); NA=not available

Income Gross domestic product per capita and health expenditure data for 1995 were given in 1995 US$ in purchasing power parities, base 1995.[15,40]

Tobacco and alcohol availability Availability of tobacco products was from tax paid data, per adult age 15 and over.[41] Cigars were omitted as they are not as strongly related to IHD as cigarettes. Alcohol, from FAO data, was given per capita.

Correlations were tested for significance by PEPI software,[42] and multiple regressions by Excel 2001.

Results

No significant correlation was found between 1995 per capita incomes and IHD or DM-1.

Ischaemic heart disease (Tables 1 and 2, Figure 1)

In Table 1, countries were ranked by IHD rate. The Japanese, ranked lowest for IHD (21.5), consumed the least milk and least saturated fat.

The next six countries with lowest IHD mortality were from central Europe or the Mediterranean. Their milk consumption was low (except for Switzerland), and A1/capita was also low. France (28.8) ranked second lowest overall for IHD; and Guernsey (40.7), where milk has been virtually A1-free for a century or more, ranked third lowest.

To test for possible under-diagnosis or under-classification of mortality to IHD, we also ranked countries by (total) CVD, and by CVD minus stroke. Correlation with IHD was r = 0.92 for CVD, and 0.91 for CVD minus stroke. For CVD, France ranked lowest, and for CVD minus stroke, second lowest; while Switzerland ranked third lowest for both, and Guernsey ranked fourth lowest for both.

The countries of North Europe, including countries they populated (with their cattle) – North America and Australasia – filled the lower half of the table and tended to consume more milk. In the last four ranks of Table 1, countries with the highest IHD rates all have more than 2 grams of A1/capita per day in their milk supply.

Tobacco and alcohol availability In 1990, neither tobacco nor alcohol was significantly correlated with IHD five years later. Tobacco product per capita sales were highest in Switzerland, and lowest in Sweden. Alcohol availability was highest in central Europe – in Germany, Austria, France and the Channel Islands – and lowest in Japan. Table 2 shows that wine supply/capita was moderately inversely correlated with IHD.

Dietary fat factors Dietary fat factors in univariate analysis (Table 2) showed significant correlation with IHD – the atherogenic index, estimated from the configuration of six dietary fats[43] (r = 0.50); and myristic, the 14-carbon saturated fatty acid. The Hegsted score (using food supply fats to estimate population serum cholesterol), saturated fat, and dairy fat were significantly correlated with IHD in 1980 and 1985, but not in 1990 and 1995.

Table 2 Correlations of supply variables, with ischaemic heart disease five years later, 1980-95, 18 countries

Mortality year	1980	1985	1990	1995				
Food, tobacco, alcohol supply 5 years prior	r	r	r	r	mean	b	95%CI	e
A1/capita supply in milk, cream g/d	0.81‡	0.76‡	0.82‡	0.76‡	1.8	25.5	14, 37	0.57
A1/capita supply in milk, cream, and cheese g/d	0.79‡	0.71‡	0.74‡	0.66†	2.8	20.6	8.3, 33	0.71
Milk protein/capita supply in milk, cream g/d	0.72‡	0.63†	0.65†	0.60†	14.7	3.2	3.1, 6.6	0.65
A1/ ß casein fraction, in milk, cream	0.30	0.39	0.43	0.36	0.43	100	-37, 236	0.54
Atherogenic index	0.73‡	0.67†	0.54*	0.50*	0.71	90	6, 172	0.78
Myristic C14:0 fat %E	0.70†	0.70†	0.60†	0.48*	1.6	64	1.2, 71	0.74
Hegsted dietary fat formula for serum cholesterol mg/dL	0.70†	0.56*	0.44	0.42	210	0.47	0.06, 1.0	ns
Saturated fat %E	0.65†	0.61†	0.45	0.37	14.2	4.6	-1.4, 10.6	ns
Dairy fat g/d	0.63**	0.62†	0.49	0.31	35.3	0.8	- 0.5, 2.2	ns
Butterfat g/d	0.61**	0.53*	0.44	0.14	10.3	0.7	- 1.8, 3.2	ns
Tobacco products without cigars g/adult/year	0.27	- 0.04	-0.38	-0.42	2380	-0.02	-0.05, 0.003	ns
Alcohol g/d	- 0.40	- 0.32	-0.29	-0.04	15.1	-0.2	-3.0, -2.5	ns
Wine g/d	- 0.59†	- 0.55*	-0.53*	-0.50*	53.0	-0.3	-0.5, -0.02	-0.17
Vegetables %E	- 0.57*	- 0.58*	-0.60†	-0.16	7.5	-3.6	-15.0, 7.8	ns
Plant foods poly-unsaturated fat (PUF) %E	- 0.79‡	- 0.82‡	-0.74†	-0.53*	1.64	-43	-79., -6	-0.87

Table 2 countries are Table 1 countries minus Guernsey and Jersey. *p <0.05; †p<0.01; ‡p <0.001; ns=not significant; r=correlation coefficient; b=univariate regression coefficient, with 95% confidence intervals; e=elasticity=% change in IHD rate related to a 1% change in the food supply variable, estimated as b*(supply variable mean/mean of 1995 IHD rate). The mean IHD rate was 80.8 for these 18 countries in 1995.

Cow milk protein supply variables Milk protein supply variables were all more strongly correlated with IHD five years later than any dietary fat variable in Table 2: A1/capita in milk and cream (r = 0.76, p <0.0005); A1/capita in milk, cream and cheese combined (r = 0.66); and cow milk protein per capita (r = 0.65). Across 18 countries studied over 20 years, of over 180 variables tested, A1/capita correlated most closely with IHD five years later. A 1% change in A1/capita in 1990 was associated with a 0.57% change in IHD mortality in 1995. Correlation between 1990 A1/capita and IHD in 1995 were stronger for male IHD (r = 0.83) than for female IHD (r = 0.69). Length of lag was not critical to the correlations. A1/capita in 1975, 1980, 1985 and 1990 was correlated with IHD in 1995 (r = 0.82, 0.76, 0.82, 0.76 respectively).

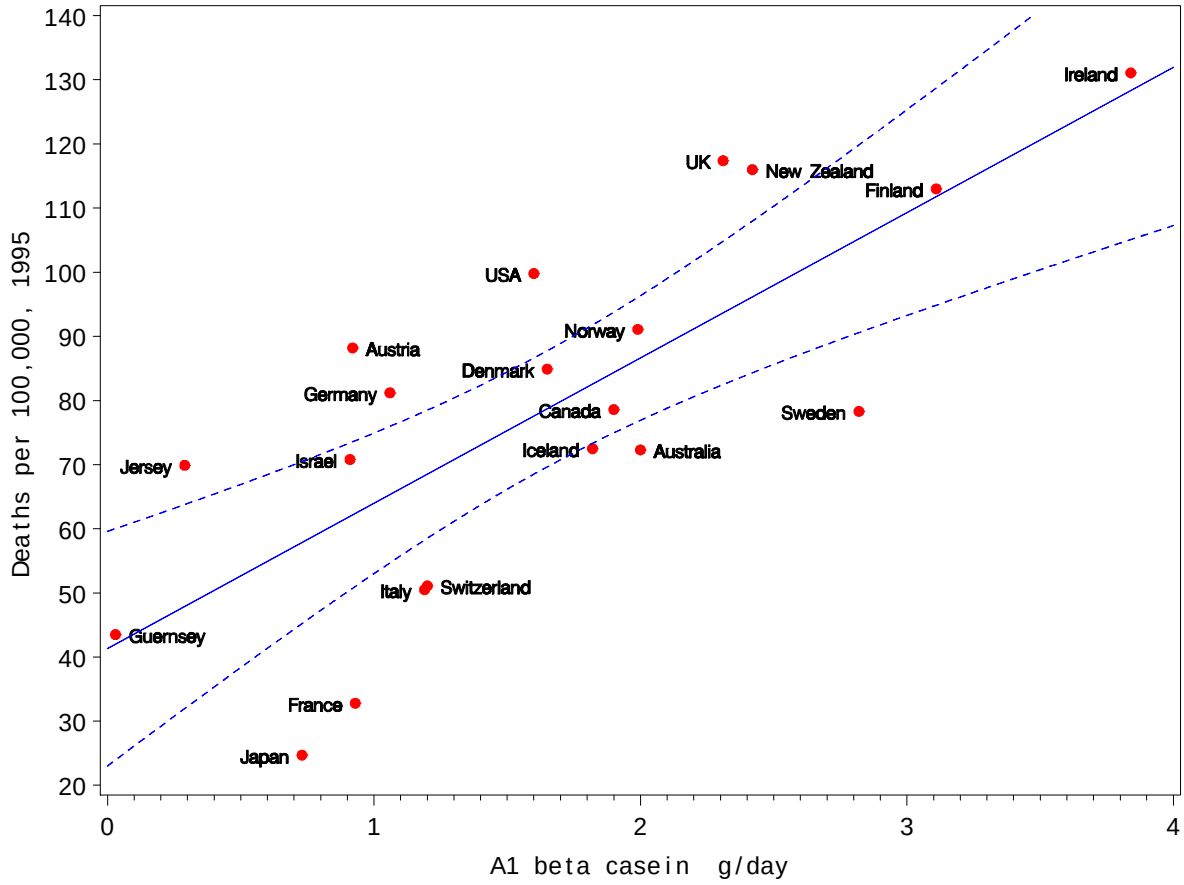
In 1995, A1/capita varied greatly among countries, from about 0.3 g/day in Guernsey, to 3.0 g/day in Finland. Between 1975 and 1995, the 20-country averages decreased for milk protein and for A1/capita by 14%, and remained the same for A1/ß; IHD decreased 57%. Most of the decreases occurred between 1985 and 1995, when

A1/capita decreased 13%, while IHD decreased more, by 37%. The correlation of the annual rates of change between A1/capita and IHD was not statistically significant.

Cheese, butter and cream, and the other caseins A1 consumed as cheese (after adjusting for imports) weakened the correlations of A1/capita in milk and cream with IHD five years later, from r = 0.76 to r =0.66. Adding A1 in cream added approximately 1% to the strength of the correlations between milk and IHD. Butter (1.1% protein by weight), in high-consuming countries, added 3–4% to estimated A1/capita, but increased the correlation of A1/capita with DM-1 by 0.001 only, and was omitted from all tables.

Per capita supply based on the B variant β-casein in milk and cream, or on any combination of A1, B and C variants of β-casein therein, or on including the A variant of kappa-casein, or including estimates of A1 in cheese, decreased correlations with IHD.

Figure 1. A1 ß-casein supply (A1/capita) 1990 and ischaemic heart disease 1995, 20 countries



r=0.76, (95% CI 0.48-0.90), p=< 0.0001. Dotted lines are the 95% confidence limits of the regression line

Analysis On multivariate analysis of the variables with highest univariate positive correlation with IHD in 1995 in Table 2, only A1/capita gave a significant result. When all 4 five-year periods in Table 2 were combined for estimating IHD five years

later, the only significant variables were the downward trend with time, as given by the calendar year, A1/capita, and plant polyunsaturated fat (PUF).

Diabetes Type 1 (Tables 3 and 4, Figure 2)

Table 3 Average supply of milk protein per capita as milk or cream in 1990–4 varied more than fourfold, from 5 g/day in Japan, to 22 g/day in Finland and Sweden.

Table 3. The per capita supply of A1 b-casein and milk protein, 1990–94, and incidence of diabetes mellitus Type 1 at age 0–14 years, 1990–94, 19 countries

Countries ranked on DM-1 incidence	**DM-1 new cases**	**Milk and cream protein per capita* g/day**	**A1b-casein fraction in milk and cream supply 1990, 1995 averaged**	**A1b-casein per capita g/day**	**DM-1 annual incidence rate per 100 000**
	n	a	b	c= a*b*0.284	
Finland	1768	21.54	0.48	2.93	36.5
Sweden	2166	22.34	0.46	2.92	27.5
Canada	204	12.18	0.52	1.79	24.2
Norway	409	14.86	0.46	1.94	21.1
UK	1111	14.20	0.53	2.14	18.4
NZ	169	14.64	0.48	2.00	17.4
Denmark	177	9.38	0.48	1.29	15.5
USA	605	14.28	0.40	1.63	14.8
Australia	722	17.19	0.43	2.12	14.5
Italy	1637	8.92	0.44	1.11	13.7
Iceland	52	17.26	0.31	1.65	13.5
Germany	903	9.65	0.43	1.18	11.0
Austria	660	15.98	0.21	0.94	9.5
Hungary	697	11.34	0.39	1.25	9.1
France	709	9.94	0.36	1.01	8.5
Switzerland	353	19.87	0.23	1.27	7.9
Israel	361	7.50	0.42	0.90	6.0
Japan	167	4.85	0.52	0.73	1.7
Venezuela	43	5.96	0.30	0.50	0.13
Total or mean	**12 913**	**13.21**	**0.42**	**1.54**	**14.3**

*fresh milk equivalents, excluding cheese and butter, including cream and yoghurt
Note: The DM-1 surveys were carried out in 1990–3 for Australia and Japan, 1989–94 for Iceland 1991-4 for Switzerland, in all others during 1990–4. Milk supply data were averaged for the same years as the DM-1 survey in that country.
Source: WHO-DiaMond Project;[10] for Iceland and Switzerland, EURODIAB ACE study group.[39]

The A1/β fraction of milk casein varied from 0.21 in Austria to 0.53 in the UK. A1/capita supply varied sevenfold, from 0.4 g/day in Venezuela to 3.0 g/day in Finland.

From 19 countries surveyed, 12 913 new cases of DM-1 were detected, with a country annual average rate of 14.3 new cases per 100 000 children age 0–14 years (boys,

14.6, girls 13.9). The DM-1 rate varied nearly 300-fold, from 0.13 in Venezuela to 36.5 in Finland.

The DiaMond Project surveys supplied age-specific DM-1 for 17 countries.[10] The country-average DM-1 rate increased from 9.3 per 100 000 at 0–4 years, to 15.9 at 5–9 years, to 18.9 at 10–14 years of age.

The correlation of milk protein with DM-1 was equally high in all age groups (r = 0.80, 0.81, 0.81) and for 0–14 years, r =0.82. The correlation with A1/capita was r = 0.91 for boys, and 0.90 for girls ($p < 0.001$), and equal at 0–4, 5–9, and 10–14 years of age. For the five Nordic countries, correlation was similarly high (r = 0.91, $p < 0.05$).

For 51 countries surveyed, DM-1 was significantly correlated with milk supply, including cheese (r = 0.70, $p < 0.001$).

Table 4. Correlations of cow proteins per capita supply with incidence of diabetes mellitus Type 1, age 0–14 years, across 19 countries, 1990–94

Cow protein variables, 1990-94 averaged	Univariate regression coefficients		Elasticity	Correlation with DM-1 incidence[§]
	b	95% CI	e	r
A1 β-casein /capita, in milk & cream	12.0	9.3, 14.7	1.29	0.92[‡]
A1+B casein/capita, in milk & cream	11.1	7.7, 14.4	1.36	0.86[‡]
A1+B +C casein/capita, in milk &cream	3.0	2.0, 4.0	1.32	0.84[‡]
A kappa-casein/capita, in milk & cream	1.8	0.9, 2.7	1.15	0.72[‡]
Protein/capita in milk & cream[¦]	1.20	0.54, 1.85	1.11	0.68[†]
A1/β casein fraction, in cheese	47.5	5.0, 90.0	1.38	0.50*
A1/β casein fraction, in milk & cream	3.1	-0.2, 6.4	0.09	0.47*
A2 β-casein /capita, milk & cream	4.8	0.2, 9.5	0.68	0.47*
A1 β-casein /capita, in cheese	9.2	0.2, 18.3	0.62	0.46*
Protein/capita, in cheese	0.57	-0.7,1.8	ns	0.23
B β-casein/capita, in milk & cream	-6.45	-30.1, 17.2	ns	-0.14
C β-casein/capita, in milk & cream	-37	-123, 48	ns	-0.22

Based on Table 3.
*$p < 0.05$; [†]$p < 0.01$; [‡]$p < 0.001$, ns = not significant
[§]DM-1 rate standardised by averaging six age-gender groups (0–4, 5–9, and 10–14 years)
[¦] For the 17 countries surveyed by DiaMond Project
r=0.82; b= univariate regression coefficient, with 95% confidence intervals; e=elasticity=% change in DM-1 rate related to a 1% change at the mean in the cow protein variable, estimated as b*(cow protein variable mean/mean of DM-1 rate)

Table 4 DM-1 at age 0–14 years was correlated with the quantity of milk and cream in the food supply, as measured by milk protein per capita (r = 0.68, $p < 0.001$). DM-1 correlated particularly with A1/capita in the food supply in the same years (r = 0.92, $p < 0.001$); but not with B and C β-casein per capita. A 1% change in A1/capita was associated with a 1.3% change (elasticity) in DM-1.

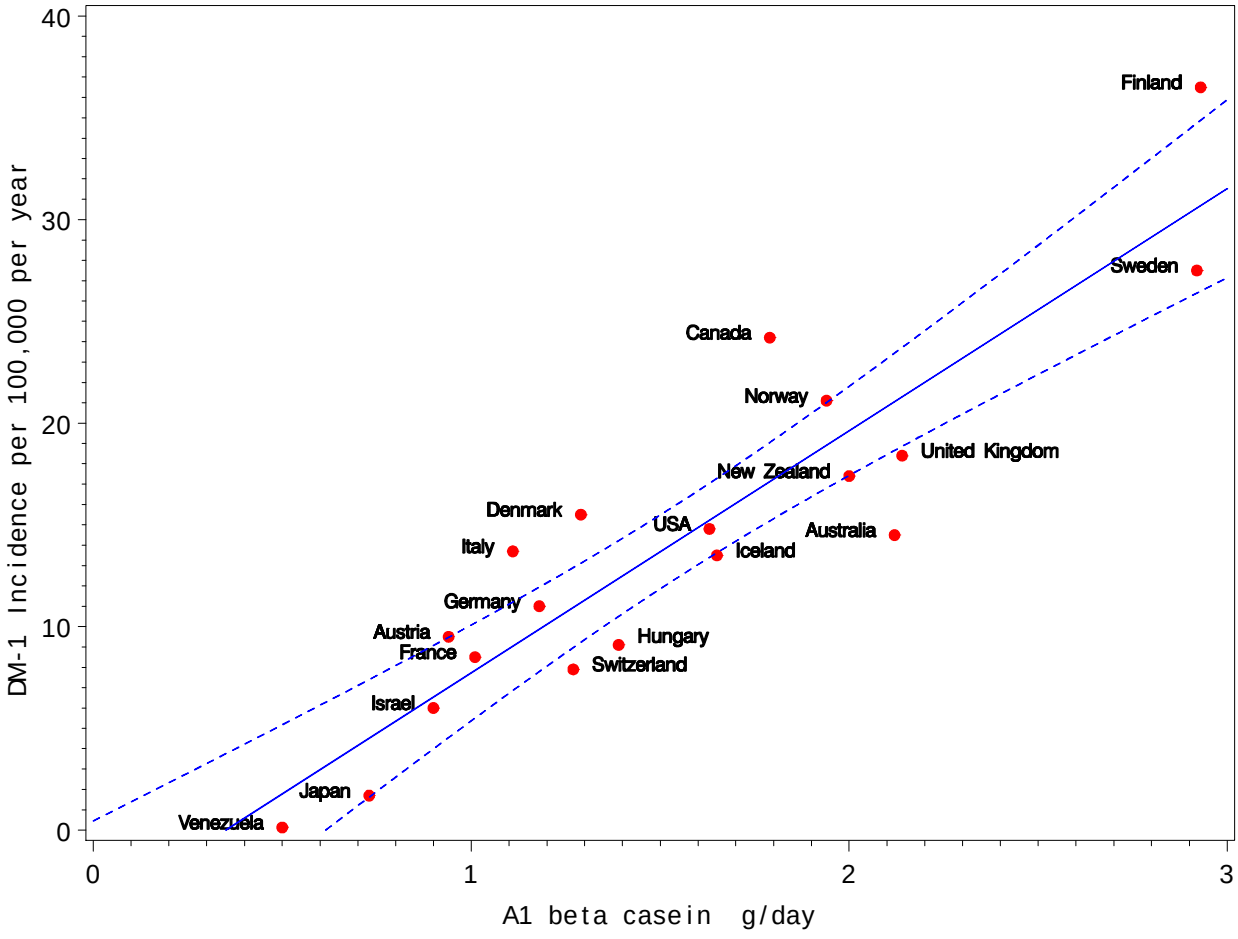
Across 75 foods and over 100 nutritive food supply variables across 18 countries, the highest positive correlation was with milk protein (r = 0.64), and with oats (r = 0.70). Latitude was significantly correlated with DM-1 (r = 0.65, $p < 0.005$), but not with IHD.

None of the genotype fractions of β-casein or of kappa-casein in milk correlated significantly with DM-1, except A1/β (r = 0.47, $p < 0.05$). The correlations of casein

genotypes were significantly inter-correlated, but among single genotypes, only A1/capita markedly exceeded milk protein per capita's correlation with DM-1. The combined A1+B, and A1+B+C per capita correlations with DM-1 were weaker than for A1/capita alone. The A variant fraction of kappa casein per capita was significantly correlated to A1/capita, and was correlated with DM-1 in the same range as total milk protein. A2 β-casein in milk protein per capita was significantly correlated with DM-1 ($r = 0.47$, $p < 0.05$), but less than total milk protein ($r = 0.68$, $p < 0.01$). A1 β-casein in cheese per capita was significantly correlated with DM-1 ($r = 0.46$, $p < 0.05$), half the $r = 0.92$ value for A1 β-casein in cow milk.

Elliott et al found a correlation ($r = 0.77$) of A1/capita with DM-1 across 10 countries based on DM-1 surveys before 1991;[6] in the present study, for 1990–4, across the same countries we confirmed this correlation ($r = 0.84$, $p < 0.005$). For the additional nine countries in this study, $r = 0.90$ ($p < 0.001$). Elliott et al found that combining per capita A1 and B β-caseins gave an improved correlation with DM-1; in this study, across the same countries, inclusion of B β-casein weakened the correlation.

Figure 2. Correlation of A1/capita (A1 b-casein in the per capita milk and cream supply) with the incidence of diabetes mellitus Type 1 at age 0–14 years of age, 1990-94, 19 countries



r=0.92 (95% CI 0.72 to 0.97) p <0.0001; dotted lines = 95% confidence limits of the regression line

Sensitivity of the methods of estimation The correlation of A1/capita in 1990 with IHD in 1995 varied from r = 0.75 for the 10 countries estimated from breed data only, to r = 0.69 for the 10 using both methods. This was due to the fact that the highest and lowest values were found in the former group. Correlating for any 19 countries out of 20, the r value varied from 0.69 (deleting Ireland) to 0.80 (deleting Australia). Deletion of the smallest countries (Jersey, Guernsey and Iceland) made no difference. Deletion of all three countries whose individual deletions most lowered the correlation (Ireland, New Zealand and Guernsey), lowered the correlation to r = 0.62.

For DM-1, removal of one country at a time from the correlation with A1/capita resulted in a correlation between r = 0.89 and 0.94 for the remaining 18 countries. Deletion of the two highest data points in Figure 2 (Finland and Sweden), lowered the correlation to r = 0.65. When the analysis was confined to the 11 countries analysed by consumer milk tests and breed-based estimates, the correlation was r = 0.69, but the consumer milk tests were all carried out approximately five years later.

Discussion

Correlation, even when statistically significant, does not prove causation, though it may raise that possibility. This is an ecological study with the limitations implied: its main value is to focus further research, and is not by itself a basis for public policy. For example, the supply of tobacco products in Table 2 was not significantly correlated with IHD five years later. This is at variance with individual-based studies – tobacco precipitates IHD mortality in tandem with atheroma, and many high-IHD populations have reduced their tobacco and saturated fat consumption. Data aggregated at national level may, however, be an efficient means of locating effects which though small for each individual are detectable in the population mean. In addition, extreme caution is required in interpreting correlations involving dietary factors, which are often inter-correlated.

Sampling errors may have occurred, due to surveying only parts of some countries for DM-1. Testing dairy herds to characterise a national herd risked local variations. Cow registration data may have over-represented high-production breeds in characterising the national herd. A single retail milk sample may not accurately characterise a country's milk for that year. Testing methods for A1/β vary somewhat. Japan lacked recent A1/β data. Israeli cows or milk were not tested; instead, tests of the United States Holsteins, from which the Israeli herd derives, were used.

A1/ß estimates were obtainable for 22 countries in total. As herd A1/ß test results may not have been representative of the national herd, we supplemented herd tests with consumer milk tests. For New Zealand, with separate herds milked for export and domestic supply up to 1992, any estimates were for the latter.

Ischaemic heart disease

In reviewing the results of the WHO MONICA (Monitoring trends and determinants in Cardiovascular disease) Project in 21 countries including New Zealand, the authors concluded that "the results support prevention policies based on the classic risk factors but suggest potential for prevention beyond these."[44] It may be timely to consider new concepts, including McLachlan's A1 hypothesis.[7]

The IHD-A1 correlation was only valid for "healthcare-affluent" countries. We assumed that a certain level of health expenditure was needed to achieve a reasonable minimum chance of survival of IHD. Per capita income was not significantly correlated with IHD mortality in the 20 countries selected, but mortality may be correlated with national expenditure on healthcare, for those with IHD surviving long enough to reach hospital. Income and IHD would have correlated significantly had we included Hungary (low GDP/capita, low health expenditure, highest IHD rate).

Similarly, the higher correlations with IHD found for A1/capita compared with other fat variables may be true only of healthcare-affluent countries. For example, inclusion of Hungary, with its atherogenic index and IHD rate each exceeding any such values in Table 1, resulted in IHD being more correlated with the atherogenic index than with A1/capita. On the other hand, low expenditure on healthcare can be expected to decrease survival and raise IHD mortality, outweighing dietary influences. Austria had a higher IHD mortality than A1/capita and dietary predictors would suggest. This was not due to misclassification of IHD (based on CVD minus stroke mortality). We were not, however, able to compare Austria with other countries for adoption of effective coronary care practices.

Food supply statistics have been significantly correlated with survey data across many countries,[45] but food supply statistics are the only feasible way to compare all countries across time. We assumed that per capita milk supply was proportional to its consumption in childhood or at age 35–64 years.

FAO assigns an average 3.3 g of fat and 3.3 g of protein per 100 g of milk. This standardised milk will give the same correlations for its protein, fat, saturated fat or any other fixed component. Table 2, however, shows that the total protein in milk was more highly correlated with IHD rates from 1980 to 1995, than was total saturated fat, total dairy fat or butter. If the milk effect was due to its dairy fat content, then the opposite should have been true.

Saturated fat recorded for individuals in the 1960s had a high correlation ($r = 0.85$) with IHD 10 years later across the 16-cohorts of the Seven Country Study.[46] Between 1970 and 1990, of the 18 countries listed for saturated fat in Table 1, the 11 English-speaking or Nordic countries, with mostly high IHD rates in 1970, all lowered per capita saturated fat in their food supply, while the other countries in Table 1 with mostly low IHD rates, increased it. This reduced the correlation between the per capita saturated fat supply and IHD in 1990, and 1995 ($r = 0.37$, Table 2).

Based on the estimated A1/capita in milk, cheese contributed an estimated country average of one third of the A1 ß-casein in the diet, and one half or more in France, Germany and Italy. Inclusion of cheese weakened the correlation with IHD of A1/capita in milk and cream by 10 percentage points (Table 2). The extent of decrease in A1/ß in manufacture or during shelf life may vary by brand or country. In any case, A1/capita in milk, whether including A1/capita in cheese or not, was more strongly correlated with IHD 5 years later than any other food supply variable found.

Plant foods (cereals, rice, nuts, beans, potatoes, olives, peas, but not counting vegetables) as measured by polyunsaturated fat (PUF), were closely associated with low national rates of IHD mortality. Of Table 1 countries, Italy, Japan and Switzerland had the highest consumption of polyunsaturated fat in plant foods.

The Mediterranean diet has been followed most closely among Table 1 countries by Italy and, though consuming less olive oil, by Israel; both countries ranked low in IHD mortality. The Mediterranean diet, as surveyed in Crete in 1948, derived half its calories from cereals, nuts and pulses, one third from olive oil, and the rest from vegetables and fruit.[47] Milk consumption was low (milk protein 8 g/day).[48] Cretan men had the lowest IHD mortality in the Seven Country Study, mostly attributed to low saturated fat. An additional explanation is that A1/capita supply on Crete was low, possibly only 0.5 g/day, due to a low milk supply, and particularly due to low cow-milk availability. Forty per cent of milk in Greece was goat or sheep milk,[49] which contains no A1 ß-casein.

The "French paradox" refers to France's low IHD mortality (second lowest, Table 1) despite a high butter supply (second highest, Table 1). This was not a misclassification error – in 1995, France also had the lowest CVD mortality, and French women had the second highest life expectancy. France's low IHD mortality has been attributed to wine, garlic, plant PUF or vitamins.[50] In France alcohol supply/capita was one half higher than in Ireland; and wine supply/capita 16 times higher (Table 1). In Figure 1, the French IHD data point is below the 95% confidence limits for A1, and high French alcohol consumption may explain this. However, alcohol was not correlated with IHD (Table 2). The Irish/French IHD rate ratio of 3.8:1 was in line with the milk protein ratios (total protein/capita, 3.1:1; A1/capita, 4.1:1) (Table 1).

The A1 hypothesis, if confirmed, could explain the low IHD rates in Mediterranean countries, and the Irish–French IHD differences.

Diabetes Type 1

Of over 170 foods and nutritional variables in the food supply in 1990, milk was the only food highly correlated ($r > 0.60$, $p < 0.01$) with DM-1, apart from the northern European crops of oats and rye, which with latitude, may merely reflect the geographical distribution of A1/capita supply.

In this 19-country study based on 1990–4 surveys, we confirmed Elliott's pre-1991 findings from 10 countries that A1/capita was highly correlated with DM-1,[6] but found that with B (or C) β-casein added, the correlation decreased, and B or C separately were not correlated with DM-1 (Table 4).

Elliott noted that the distinctive peptide formed mostly from A1 β-casein and partly from B β-casein was β-casomorphin-7, and this was possibly the active ingredient. Lack of correlation between DM-1 and B β-casein raises the possibility that B β-casein, which differs in solubility, may be processed differently by the intestinal mucosa. Countries with above-average B β-casein were Australia, Austria, Denmark, France, Germany and Venezuela. Milk may be exposed to different temperature patterns at the farm, or during processing, across countries and time periods.

The method used here and by Elliott[1] assumed that childhood milk consumption was proportional to its per capita supply. Surveys have since confirmed that A1 β-casein consumption of two-year-old children was lower in Iceland (1.7 g/day) than in other Nordic countries (average 2.4 g/day).[51] No such difference was found in surveys of 11–14 year-olds.[51] The correlation between DM-1 and A1/β was equally high at 0–4, 5–9 and 10–14 years of age, suggesting that early childhood exposure to cow milk

A1/β may permanently change the islet cells, making them prone to other factors or processes that cause islet cells to die at a later age. Anti-A1 antibodies tend to be higher in DM-1 diabetics and their siblings, while anti-A2 antibodies tend to be higher in their parents and controls. This suggests a defective immunotolerance to cow milk antigens in DM-1,[52] possibly due to ß-casomorphin-7.[53]

The A variant of kappa casein/capita (Table 4) was highly correlated with A1/capita (r = 0.79), and less so with DM-1. Genes for kappa and beta casein are situated very close together on cattle chromosome 6.[54] Besides A2, B and C β-casein, other cow proteins in the milk supply – albumin, immunoglobulin, and lactoferrin – showed no correlation with DM-1 in Nordic countries.[51]

A1 β-casein in cheese per capita (estimated from A1/ß of the milk) did not correlate with DM-1, certainly not as closely as A1 casein in milk and cream. First, child consumption of cheese, more than milk, was likely to vary from adult consumption. Second, due to wastage, cheese supply may not reflect consumption as does milk supply. Third, its A1/ß ratio was likely to vary in ways not predicted by the A1/ß of the milk it was made from. Proteolytic enzymes, salts, temperature during manufacture, and on-shelf ageing,[55] can vary the A1/ß ratio between types of cheese. Information on the market share and on-shelf A1/ß tests for each cheese type might improve the estimate of national A1/capita from cheese as consumed.

Insulin-dependency makes for a clear definition of DM-1, and diabetic registers and the second round of the DiaMond survey have made for very high ascertainment. The increase in DM-1 rates with age during childhood suggested environmental causes. From 1960 to 1996, A1/capita across 17 countries with historical data declined 21%, or 0.6% a year, whereas the rate of DM-1 increased by an average 3% a year in 37 populations surveyed.[9] Other factors besides A1/capita and milk supply per adult are needed to explain the global **increase** in DM-1 in **children**. While the milk supply has decreased, child nutrition surveys are needed to determine whether rising incomes and the marketing of infant formula, coloured and flavoured milks, yoghurts, and ice cream may have led to increased children's consumption of cow protein, and thereby increased A1/capita or ß-casomorphin-7 in their diet.

A DM-1 rate was not calculated for Guernsey for Table 3, as only five DM-1 cases were found in 1990–4, and we had no information on genetic predisposition to DM-1 among Guernsey children. Clearly, however, milk very low in A1 does not entirely prevent DM-1 from occurring. Similarly, Jersey residents consumed only Jersey milk (A1/β = 0.09, A1/capita 0.3 g/day), and again numbers were small.

The low Venezuelan DM-1 rate may reflect incomplete ascertainment but, even assuming it was 10 times higher, the correlation over all countries was unaltered at r = 0.92. DM-1 in countries excluding Australia had a correlation with A1/capita of r = 0.94; Australian DM-1 data were based on one survey site in New South Wales.

In summary, from 1980 to 1995, IHD mortality in 20 healthcare-affluent countries was more highly correlated with total milk proteins (and particularly A1) than with fats in the food supply at population level, providing an alternative and testable hypothesis to explain the higher IHD mortality in northern compared to southern Europe.

Across 51 countries surveyed, DM-1 rates were significantly correlated with per capita fresh milk protein, and in 19 countries for which data were available, A1 β-casein/capita substantially increased this correlation, from 68% to 92%. In contrast, A2, B and C variants of β-casein in milk, and cheese proteins, correlated less strongly with DM-1 than total milk protein.

The correlations of A1/capita with DM-1 and IHD rates raise the possibility that intensive breeding of cows over many years may have emphasised a genetic variant of milk with adverse effects in humans. Clinical trials will be needed to determine whether A1-free milk can reduce the risk of DM-1 and IHD.

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