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Type I (insulin-dependent) diabetes mellitus and cow milk: casein variant consumption

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Summary Previously published Type I (insulin-dependent) diabetes mellitus incidence in 0 to 14-year-old children from 10 countries or areas was compared with the national annual cow milk protein consumption. Countries which were selected for study had appropriate milk protein polymorphism studies, herd breed composition information and low dairy imports from other countries.

Total protein consumption did not correlate with diabetes incidence ($r = + 0.402$), but consumption of the β-casein A^1 variant did ($r = + 0.726$). Even more pronounced was the relation between β-casein ($A^1 + B$) consumption and diabetes ($r = + 0.982$). These latter two cow caseins yield a bioactive peptide β-casomorphin-7 after in vitro digestion with intestinal enzymes whereas the common A^2 variant or the corresponding human or goat caseins do not.

β-casomorphin-7 has opioid properties including immunosuppression, which could account for the specificity of the relation between the consumption of some but not all β-casein variants and diabetes incidence. [Diabetologia (1999) 42: 292–296]

Keywords Milk, casein, epidemiology, immunosuppression, beta-casomorphin.

Several observations have linked the great global differences in childhood diabetes incidence to national cow milk consumption [1–3]. Milk is a multi-nutritional food consisting of protein, fat and carbohydrate components. Milk protein can be broadly divided into whey protein and casein protein, and one of the major casein proteins is β-casein. This protein has a number of genetic variants of which the A^1 and A^2 variants are the commonest in most cow breeds [4].

As there appeared to be some notable exceptions to the generalisation that the more milk consumed in a country the higher the diabetes incidence, we examined the composition of milk as well as its consumption together with diabetes incidence wherever this was possible.

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Methods

Sources of data. Data for the genetic polymorphism and for breed composition of national dairy herds were from published data [5–18] and personal communications with Gerd Vegurad (Agricultural University of Norway, N-1432 Aas) and Ólafur Reykdal (University of Iceland, IS-101 Reykjavik). Data for the dairy protein available for consumption were obtained from the FAOSTAT database [19]. Data for the incidence of Type I (insulin-dependent) diabetes mellitus over various eras between 1960 and 1991 were from the WHO/DIAMOND study [20] and included its data sources for the individual countries reported in this study.

Selection of countries. Countries were selected that had a complete set of data for breed composition and for milk protein polymorphism that were representative for the country or for the particular region of the country reported in the diabetes study. Countries were not selected if their polymorphism data were for only unique or minor breeds of cow, or their data were for only a small sample population of cows, or they had not phenotyped all β-casein variants. In addition, countries were not selected if they had a high proportion of imported dairy foods since β-casein variants consumption could not be accurately ascertained. The countries and regions selected

Table 1. Weights used for calculation of the weighted mean

Weight used	Year that weight was applied	Age group of children[a]	Assumptions
15	Each year of diabetes study	0–14	Equal number of children of each age The same amount of milk is available to each age group
14	Study period start date minus 1 year	1–14	
13	Study period start date minus 2 years	2–14	
and so on down to:			
2	Study period start date minus 13 years	13–14	
1	Study period start date minus 14 years	14	

[a] Based on age of children at the start of the diabetes study

Table 2. Data for Type I diabetes in 0 to 14-year-olds, total milk protein and casein variant consumption by country

Country	Diabetes study period	Diabetes incidence rate over study period (per 100 000 pa)	Mean milk protein consumption (grams per day per person)		β-Casein frequency		β-Casein consumption				% Dairy Imports (over diabetes study period)
			Non-weighted	Weighted	A^1	B	Non-weighted A^1	Weighted A^1	Non-weighted $A^1 + B$	Weighted $A^1 + B$	
Australia	1985–1989	13.2	22.6	21.9	0.310	0.170	1.990	1.929	3.081	2.987	3.1
Canada	1971–1985	9.8	17.5	18.2	0.520	0.027	2.589	2.686	2.725	2.828	4.3
Denmark	1989–1990	21.5	22.0	19.6	0.492	0.094	3.075	2.735	3.662	3.258	4.9
Finland	1987–1989	35.3	26.7	26.7	0.510	0.001	3.862	3.870	3.867	3.876	1.5
Germany	1960–1989	7.4	17.3	17.3	0.392	0.062	1.930	1.930	2.236	2.236	8.2
Iceland	1970–1989	9.4	34.2	34.6	0.250	0.000	2.425	2.453	2.425	2.453	0.1
New Zealand	1978–1985	9.8	21.0	20.8	0.293	0.156	1.749	1.734	2.681	2.658	0.2
Norway	1989–1990	20.8	24.0	26.0	0.460	0.018	3.135	3.401	3.258	3.534	1.4
Sweden	1978–1987	24.4	29.6	28.2	0.463	0.008	3.895	3.714	3.962	3.778	4.4
USA, San Diego	1978–1981	9.4	20.5	20.9	0.370	0.045	2.154	2.201	2.416	2.468	5.1

were Australia, Canada, Denmark, Finland, Germany, Iceland, New Zealand, Norway, Sweden and San Diego, USA.

Method of calculation of consumption. Consumption of β-casein variant was calculated according to the equation:

$$C = (f \times B) \times P \times Y$$

where
C = consumption of β-casein of the particular variant(s);
f = frequency of the particular β-casein allele(s) in a breed in the national dairy herd;
B = the proportion of the breed in the national dairy herd;
P = the mean daily intake of dairy protein (from FAO data);
Y = the fraction of β-casein as a proportion of all the protein in milk

The value of Y was taken as 0.284 [21].

The mean daily intake of dairy protein (P) was calculated using two methods. A non-weighted mean was obtained by calculating the mean protein consumption over the particular period of observation of diabetes incidence reported for each country. This type of mean does not take into account the consumption of milk protein in the years prior to the diabetes study. Therefore, a weighted mean was calculated by applying a decreasing linear weight to the milk protein consumption in the years prior to and during the diabetes study. The weights were based on the number of people available to consume milk in each of the years prior to and including the years of the study (Table 1). This type of weighting assumes an accumulative dose response between diabetes and the protein intake.

Table 3. Spearmans rank correlation coefficients for Type I diabetes compared with mean protein and casein consumption

Correlation coefficients	*r*	Level of significance
Non-weighted		
Total milk protein	+ 0.537	11 % (Not significant)
Casein A^1	+ 0.774	0.8 %
Casein A^1 + B	+ 0.982	0.01 %
Weighted		
Total milk protein	+ 0.402	25 % (Not significant)
Casein A^1	+ 0.726	2 %
Casein A^1 + B	+ 0.982	0.01 %
Excluding Iceland (weighted)		
Total milk protein	+ 0.736	2 %
Casein A^1	+ 0.770	1.5 %
Casein A^1 + B	+ 0.979	0.01 %

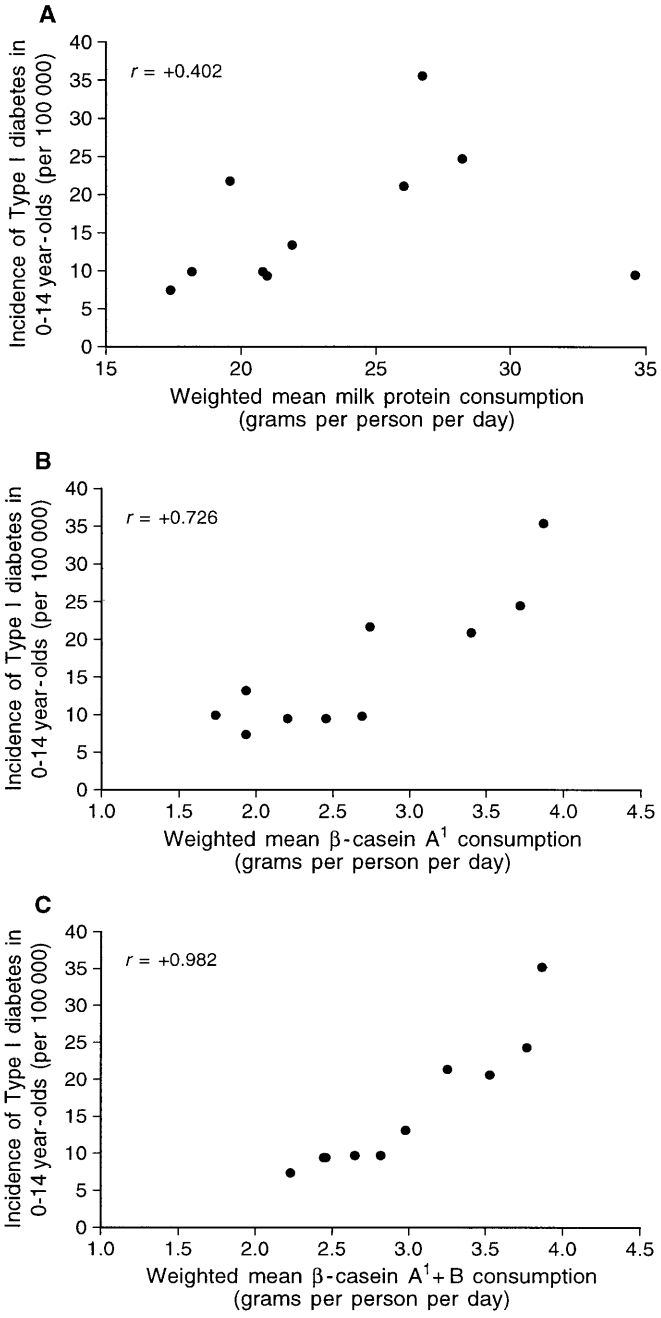

Fig. 1 A–C. Incidence of Type I diabetes in 0 to 14-year-olds with different types of milk protein consumption

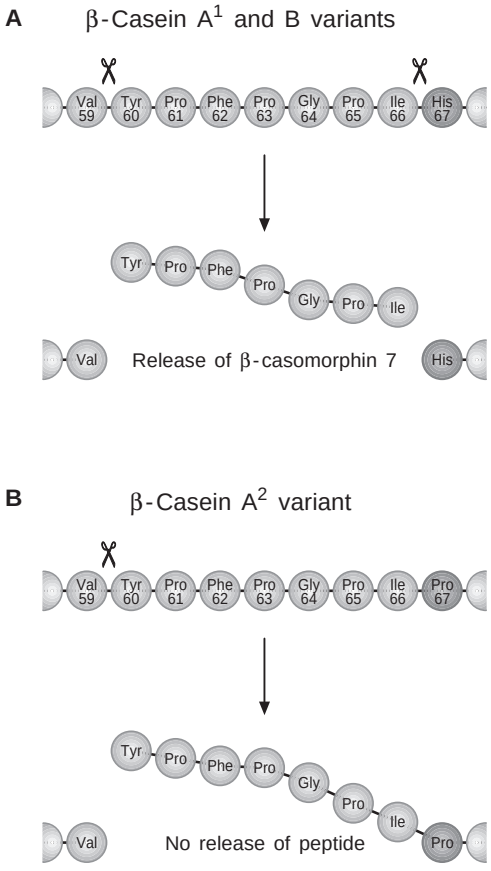

Fig. 2 A, B. Release of β-casomorphin-7 from β-casein variants. **A** Release of β-casomorphin-7 from β-casein A^1 and B variants. The A^1 and B variants have a histidine at position 67 that determines the enzymatic cleavage of the peptide bond releasing β-casomorphin-7. **B** The A^2 variant does not cleave due to the presence of a proline at this position

Results

Table 2 gives a summary of the data for diabetes incidence and the data for total milk protein and β-casein variant consumption for each country. There was not much variation between the values calculated for the weighted and non-weighted means of total milk protein consumption for most countries. Denmark and Norway had the greatest differences between the two calculated means (more than 2 grams a day for each person), as in those two countries there were large fluctuations in milk protein consumption in the period just before and during the time the diabetes data was collected.

Spearman's rank correlation coefficients (which are appropriate for these data) were calculated for diabetes incidence compared with mean total protein and β-casein variant consumption (Table 3). There was no significant correlation between either weighted or non-weighted total milk protein and incidence of diabetes. When β-casein A^1 consumption was tested there was a marked increase in r value which was significant at 0.8 % for the non-weighted mean and at 1.5 % for the weighted mean values. When β-casein A^1 plus B variants were tested, there was a further increase in the r value which was significant at the 0.01 % level for both means.

Figure 1 shows the plots of Type I diabetes compared with milk protein and β-casein variants consumption. An unusual point was apparent in Figure 1A that added some leverage to the overall correlation coefficient for total milk protein consumption compared with Type I diabetes. This point was Iceland. We therefore re-tested the correlation coefficients with the Icelandic data excluded (Table 3).

Discussion

Previous studies have shown a strong correlation between the consumption of milk and the incidence of diabetes [1–3]. Countries that had a low milk consumption such as Japan had a low diabetes incidence, whereas those with a high consumption such as Finland, had a high diabetes incidence. Our study, on the other hand, has focussed on the consumption of only the protein component of milk that was available in both primary (liquid milk) and in secondary (milk products, ingredients, etc.) forms. Furthermore, the consumption of specific milk proteins that are known to yield bioactive peptides [22, 23], were tested.

The A^1 and B variants of β-casein have a histidine at position 67 that determines the enzymatic cleavage of the molecules yielding β-casomorphin 7 (Fig. 2) [23]. The A^2 variant does not cleave at this position due to the presence of a different amino-acid (proline). β-casomorphin-7 has opioid properties [24], and has been shown to inhibit human intestinal lymphocyte proliferation in vitro [25]. It is possible that such an immune suppressant influences the development of gut-associated immune tolerance, or suppresses defence mechanisms towards enteroviruses [26], both of which have been implicated in the aetiology of Type I diabetes. Other immunosuppressive effects which might contribute to diabetes include activation of endogenous retroviruses associated with the disease [27].

The countries used in this study were selected only if they could provide appropriate and reliable data for the calculation of β-casein variant consumption and for the incidence of diabetes. The selected countries do not therefore represent a random sample of the global population. Caution needs to be taken when drawing conclusions from such samples. Nevertheless, the progressively increasing statistical significance of diabetes incidence related to total protein consumption, then β-casein A^1 and then β-casein ($A^1 + B$), together with a plausible mechanism of pathogenesis warrant further investigation of the role of these milk proteins in diabetes. Clearly not all milk is the same and milk from different countries can vary considerably in the composition of milk protein variants.

Excluding Iceland from the data analysis substantially increased the level of statistical significance for total milk protein consumption compared with Type I diabetes (Table 3). Iceland was an unusual point because it had both the highest milk protein consumption and the lowest A^1 and B values out of the selected countries. The strong correlation between total milk protein consumption and Type I diabetes (when excluding Iceland) agrees with the findings from other studies [1–3]. The relatively low proportion of β-casein A^1 in Icelandic milk could account for the low incidence of childhood diabetes despite their very high consumption of milk. Anecdotally, the Maasai people of Africa have had until recent times a very large intake of cow milk from early infancy [28], yet a very low incidence of diabetes in childhood (Dr M. Jacobson, Doctor in Charge, Selian Lutheran Hospital, Arusha, Tanzania, personal communication.). Their herds consisted of *Bos indicus* cows which produce a low protein milk containing predominantly β-casein A^2 [4].

In view of the findings in this study, there is a necessity to consider not just the role of total milk consumption or its time of introduction into the weaning diet, in the aetiology of Type I diabetes, but also the role of individual milk protein variants. The ecological associations shown in this report have a high degree of specificity and statistical significance. The absolute specificity of the biological origin of the immunosuppressant β-casomorphin-7 together with the possible role of this peptide in diabetes causation add considerably to the plausibility of the statistical association shown being causal.

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