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A multi-centre, blinded international trial of the effect of A^1 and A^2 β-casein variants on diabetes incidence in two rodent models of spontaneous Type I diabetes

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Abstract

Aims/hypothesis. The diabetes-inducing potential of cows' milk is still debated and there is no consensus on the diabetogenicity of individual milk proteins. A^1-β-casein has been associated with increased diabetes frequency in ecological studies and in NOD mice. Our aim was to ascertain whether A^1-β-casein was more diabetogenic than A^2 and to test the diabetogenicity of a milk-free diet in animals representing different forms of spontaneous Type I (insulin-dependent) diabetes mellitus.
Methods. Defined diets were coded and shipped to laboratories in New Zealand (NOD/NZ), Canada (BB) and the UK (NOD/Ba). Base diets were Pregestimil (PG) and ProSobee (PS). Purified fractions of whole casein (WC), A^1 or A^2-β-casein were added at 10%. A milk-free, wheat-predominant, NTP-2000 diet was the control. Animals were fed from weaning up to 150 or 250 days, and insulitis, diabetes frequency and expression of pancreatic cytokines were assessed.
Results. Diabetes incidence was highest in three locations in animals fed NTP-2000. PG and PS diets were protective except for NOD/Ba mice fed PG+WC where incidence was similar to NTP-2000. A^1 and A^2 diets were protective in both models, but A^1 β-casein was slightly more diabetogenic in PS-fed BB rats. The New Zealand study was confounded by an infection.
Conclusion/interpretation. A milk-free, wheat-predominant diet was highly diabetogenic in three widely separate locations in both animal models. A previous result that A^1 β-casein was more diabetogenic than A^2 β-casein in NOD mice was not confirmed; both β-casein variants were protective in BB rats and NOD mice. Whole Casein promoted diabetes in NOD/Ba but protected BB showing that unique diabetes haplotypes react differently to dietary proteins. A^1- was more diabetogenic than A^2-β-casein only in PS-fed BB rats. Neither the analysis of insulitis nor of pancreatic cytokine gene expression showed a difference between A^1 or A^2 β-casein fed animals. Milk caseins are unlikely to be exclusive promoters of Type I diabetes, but could enhance the outcome of diabetes in some cases. Other diet components such as wheat could be more important promoters of Type I diabetes. [Diabetologia (2002) 45:1240–1246]

Keywords Environment, diet, NOD mice, BB rats, milk, food, nutrient, pancreas, cytokine, pathogenesis.

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Abbreviations: BB, BioBreeding; NOD, non-obese diabetic; BCM-7, β -casomorphin-7; FAD, Food and Diabetes Trial; PG, Pregestimil; PS, Prosobee; WC, whole casein; NZDRI, New Zealand Dairy Research Institute; Tyr, tyrosine; Gly, glycine

Type I (insulin-dependent) diabetes mellitus is an autoimmune disease affecting approximately 0.2 to 0.6% of the population of developed countries [1]. This form of diabetes is thought to result from an interaction among several risk genes and environmental factors that activates immune cells capable of destroying the insulin-producing beta cells in the pancreatic islets. The exact identity of the full complement of diabetes risk genes is not known and the genes most closely linked to diabetes also commonly occur in people who do not develop diabetes during their lifetime. Identical twins are only 30 to 40% concordant for the disease, and incidence of diabetes varies considerably among countries suggesting that environmental factors have an important effect on disease development [2]. A number of environmental factors have been implicated in diabetes pathogenesis including viral infections [3], exposure to chemicals [4] and certain types of food [5]. Of the food types, cows' milk has received special attention because it is introduced early into the diet as a supplement or a replacement for breast-milk and also because of its continued, frequent consumption throughout life. Nearly 19 years after the first reports linking milk and Type I diabetes, its role in promoting human diabetes remains controversial [6, 7, 8]. There are contradictory studies showing both an association between early exposure to cows' milk [5, 7], as well as others showing no influence [8, 9, 10, 11]. Other foods could also play a role in diabetes pathogenesis. At a recent international meeting on this issue, it was concluded that there is nothing unique about cows' milk and that a number of different food types might be diabetogenic [12]. It could be that some susceptible people are unable to handle commonly encountered dietary antigens in a normal manner.

The milk from humans and cows differs in several qualitative and quantitative aspects: human milk is low in protein (0.9 vs 3.4 g/100 g) and contains 30% compared with 80% casein in cows' milk. Previous work found that milk protein induced diabetes in non-obese diabetic (NOD) mice and it was proposed that this effect was attributable to the A^1 β-casein variant rather than the A^2 variant [13]. An association between the incidence of diabetes in children younger than 14 years of age and the consumption of β-casein A^1 and B was later reported [14]. In addition, a group from Iceland recently reported that β-casein fractions could influence rates of Type I diabetes [16]. It was proposed that the diabetogenic effect of A^1 β-casein was attributable to the release of β-casomorphin-7 (BCM-7) during digestion [14]. BCM-7 is a bioactive peptide which influences the function of immune cells. Studies in vitro have shown that BCM-7 can only be released from β-casein variants A^1, B and C and not from β-casein variants A^2 or A^3. However, confirmation that BCM-7 is released in vivo could not be obtained [15]. Although BCM-7 was shown to produce different effects when incubated with immune cells from pre-diabetic and normal human subjects [13], the link, if any, between this peptide and diabetes incidence is not clear.

Since ecological analyses and results from studies of NOD mice [13] indicated that A^1-β-casein might be more diabetes-promoting than A^2-β-casein, it was important to clarify to what extent these protein variants promote the development of spontaneous diabetes. We carried out this blinded, multinational, multi-centre long-term feeding trial of defined protein fractions fed to NOD mice and BB rats, the Food and Diabetes (FAD) Trial, to evaluate the diabetogenicity of purified A^1-β-caseins or A^2-β-caseins. A further objective of this trial was to investigate the diabetogenic effect of a predominantly wheat-based, milk-free diet.

Materials and methods

Animals. Three animal colonies were involved in the feeding experiments. NOD/Ba mice were from the Department of Diabetes and Metabolism, St. Bartholomew's Hospital, London, UK, where they have a stable diabetes incidence of approximately 70% in females by age 30 weeks and are maintained in standard conditions. NOD/NZ mice from the Department of Paediatrics, University of Auckland traditionally showed a lower diabetes frequency of approximately 40% by week 30 and were maintained in standard conditions. The BB rat colony at Health Canada in Ottawa is descended from the original animals housed at BioBreeding Laboratories in Ottawa where the (diabetes-prone) BioBreeding (BB) rat was first discovered. The animals are maintained in specific-pathogen-free conditions and the diabetes incidence has remained stable at approximately 60 to 70% for several years. The animals in each of the three colonies had free access to standard cereal-based laboratory rodent diets and tap water. Animals from individual litters were distributed randomly across all experimental groups. There were 35 NOD mice and 30 BB rats per group; 4 animals were removed from each group at an early stage of disease development when diabetes incidence was approximately 15% in the control group, to allow further analyses of pancreas cytokine patterns. Animals were cared for according to international regulations of laboratory animal care and handling approved by the animal care committee at each institution.

Diets. Diets were introduced at weaning at approximately 17 to 21 days of age for NOD mice and 23 days for BB rats. Feeding continued until 250 days for the mice and 150 days for the rats. Pregestimil and ProSobee (Table 1) were purchased from Mead Johnson (Auckland, New Zealand). Casein components were made from either β-casein A^1/A^1 or A^2/A^2 phenotype milk at the New Zealand Dairy Research Institute (NZDRI) pilot plant [13]. The purity of the β-casein phenotype milks and lactic caseins produced from these milks was validated using acid urea polyacrylamide gel electrophoresis [17]. Blending of the diets was carried out in the NZDRI pilot plant and all test diets were coded, shipped and used blind. Diet codes were held at the NZDRI and also independently by Dr. J. Norris, Denver, Colorado, USA. The codes were only shown to the investigators at the end of the study. Test materials were added as a 10% fraction of either base diet as follows: (i) Pregestimil (PG), a hydrolysed casein based formula; (ii) ProSobee (PS), a soy isolate based infant formula; (iii) Pregestimil plus 10% whole ca-

Table 1. Macronutrients and major components of Pregestimil and ProSobee

Selected components	Pregestimil [30]	ProSobee [31]
Macronutrients (g·100 g^{-1} powder)		
Protein	14	13
Fat	28	27
Carbohydrate	51	54
Major components (%)		
Corn syrup solids	32	55
Vegetable oils	29[a]	27
Soy protein isolate	0	15
Casein hydrolysate	17	0
Dextrose	10	0
Modified corn starch	8	0
Added micronutrients	<2	<1

[a] Includes 16% Medium chain triglycerides (MCT oil)

sein (PG+WC); (iv) Pregestimil plus 10% casein containing only the β-casein A^1 variant (PG+A^1); (v) Pregestimil plus 10% casein containing only the β-casein A^2 variant (PG+A^2); (vi) ProSobee plus 10% whole casein (PS+WC); (vii) ProSobee plus 10% casein containing only the β-casein A^1 variant (PS+A^1); (viii) ProSobee plus 10% casein containing only the β-casein A^2 variant (PS+A^2); (ix) NTP 2000 certified rodent diet (cereal based, milk-free).

The NTP-2000 diet (Zeigler Brothers, Gardners, Pa., USA), is an open formula (% ingredients known), non-purified diet for rodents developed by the U.S. National Toxicology Program of the National Institute of Environmental Health Sciences. NTP-2000 does not contain any milk protein. This is a mainly plant-based diet with wheat as the major component (37%), followed by corn, soybean meal, alfalfa meal, oat hulls, fish meal and cellulose. The diet contains approximately 14.6% protein (from wheat, soybean, corn, alfalfa and fishmeal but no milk proteins), 8.2% fat, 9.9% crude fibre, 52% carbohydrate, 10.7% moisture; the remainder is native and added micronutrients. The NTP-2000 diet used in these studies was irradiated, and contained low amounts of chemical and microbial contaminants. The diet is designed to provide consistent ingredient composition, decreased protein, low concentrations of environmental contaminants, and in long-term tests with rats was associated with decreased nephropathy, leukaemia, slower growth of mammary tumours, and prevention of nephrocalcinosis [18].

Pancreas inflammation. Insulitis was evaluated by examining 5 µm sections of pancreatic tissue stained with hematoxylin and eosin. A rating was then assigned based on the severity of lymphocytic infiltration and destruction of the pancreatic islets as reported previously for NOD mice [19] and BB rats [20].

Determination of diabetes. Initial screening for diabetes was made by analysis of glucose in the urine, a reading of 2+ or greater using Testape was considered positive. Diabetes was confirmed if blood glucose concentrations exceeded 12 mmol/l (214 mg·dl^{-1}).

Reverse transcriptase PCR analysis of pancreas cytokines. Four BB rats and NOD mice from each group were killed between 79 to 80 days of age and 70 to 90 days of age respectively. The pancreas was removed and total RNA was isolated using Trizol Reagent (Life Technologies, Karlsruhe, Germany) according to the manufacturer's instructions. Reverse transcription of RNA into cDNA was carried out [21]. Real time quantitative PCR was done for the analysis of mouse cDNAs using primers for β-actin, IFN-γ, IL-10, and TGF-β [22] and slightly modified. Briefly, PCR reactions were run in a total volume of 40 µl containing 2 to 4 µl cDNA, 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 8.3), 10 mmol/l EDTA, 60 nmol/l Passive Reference 1200 µmol/l dATP, dCTP, dGTP and 400 µmol/l dUTP, $MgCl_2$ (9 mmol/l for β-actin and TGF-β, 5 mmol/l for IFN-γ, and 7 mmol/l for IL-10), 100 to 200 nmol/l of each primer and the corresponding detection probe, and 0.625 U AmpliTaqGold (PE Biosystems, Weiterstadt, Germany). Each amplification was done in triplicate.

Analysis of rat cDNAs was carried out using a semi-quantitative method [21] and primer sequences for rat IFN-γ, IL-10, TGF-β, and β-actin were obtained from Clontech (Palo Alto, Calif., USA). To obtain semi-quantitative results, the linearity between the amount of cDNA and signal intensity of the PCR products must be calculated. Therefore, serial dilutions of each cDNA were used. In all amplifications, template-negative controls confirmed the absence of contaminating cDNA. Amplificates were visualised in a 1.5% agarose gel containing ethidium bromide. For quantification, the fluorescence intensity of each band was calculated as Boehringer Light Units (BLU) using a Lumi-Imager (Boehringer Mannheim, Mannheim, Germany) and analysis software (LumiAnalyst 3.0, Boehringer Mannheim). For each cDNA, a curve was generated by plotting the amount of cDNA in the respective dilution versus the corresponding BLU. Linear regression was carried out with this curve and the mRNA amount was set as the BLU value for 1 µl cDNA. The correlation between different amounts of cDNA and the corresponding fluorescence intensity ranged between 0.90 and 0.99. For both PCR methods, the relative amounts of cytokine mRNA were expressed as arbitrary units as the ratio of cytokine to the respective β-actin mRNA.

Statistical analyses. Survival analyses were carried out using Kaplan-Meier and log-rank tests. Insulitis, body weights and cytokine mRNA levels were compared using a one-way ANOVA. Differences were considered statistically significant if the *p* value was equal to or less than 0.05.

Results

In the UK (NOD/Ba) section of the trial, diabetes was less frequent in all of the test groups compared with NTP-2000-fed control animals, except for the PG+WC group which was not different from the NTP-2000 group. Whole casein was less protective than casein fractions with PS+WC associated with more diabetes than PS alone, PG+WC>PS+A^2; these differences were statistically significant. There were no other significant differences (Table 2, Fig. 1).

In the Canadian (BB rat) section of the trial, rats fed NTP-2000 developed more cases of diabetes than rats fed all other diets. PS+A^1 produced more diabetes than PS+A^2 (p<0.05). There were no other significant differences (Table 2; Fig. 2).

The finding of more diabetes cases in the NTP-2000 control group was also observed in the New

Table 2. Final incidence of diabetes in NOD/Ba mice and BB rats and statistical significance of differences in diabetes incidence

Diabetes incidence

	Control NTP-2000	PG	PG+WC	PG+A1	PG+A2	PS	PS+WC	PS+A1	PS+A2
NOD/Ba	22/31 (71%)	11/31 (36%)	18/31 (58%)	10/30 (33%)	11/29 (38%)	5/30 (17%)	14/30 (47%)	9/30 (30%)	9/28 (32%)
BB	19/26 (73%)	10/26 (39%)	9/26 (35%)	7/26 (27%)	9/26 (35%)	10/26 (39%)	7/26 (27%)	12/26 (46%)	5/26 (19%)

Statistically significant differences[4]

Control NTP-2000					
PS+A1	1, 2, 3	–			
PS+A2	1, 2, 3	2	–		
PG+A1	1, 2	3		–	
PG+A2	1, 2				–
	Control NTP-2000	PS+A1	PS+A2	PG+A1	PG+A2

[1] Statistically significant with NOD/Ba
[2] Statistically significant with BB
[3] Statistically significant with NOD/NZ
[4] Comparisons significant at $p \leq 0.05$

NB: NOD/NZ data collection limited due to *Clostridium* sp. infection
PS ProSobee, *PG* Pregestimil, *WC* addition of 10% Whole β-casein, *A1+A2* addition of 10% β-casein A1+β-casein A2 fractions respectively

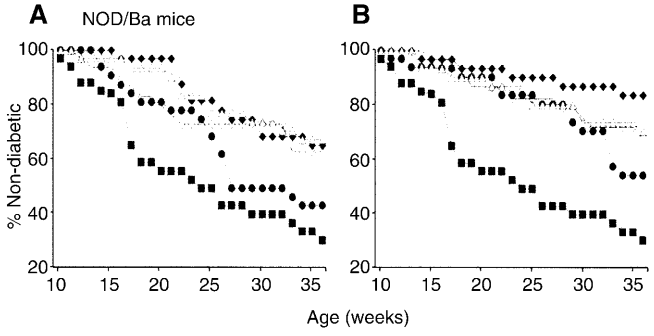

Fig. 1A, B. NOD/Ba mice survival on various diets. NOD/Ba mice were fed control, cereal-based NTP-2000 or semipurified diets in which Pregestimil or ProSobee base was supplemented with 10% defined milk fraction. Animals were fed from weaning to 250 days; *n*=28–31/group. **A** Pregestimil base: Control NTP-2000 (*closed squares*), Pregestimil (PG) (*closed diamonds*), PG+Whole Casein (*closed circles*), PG+A1-β-Casein (*open triangles*), PG+A2-β-Case (*open diamonds*). **B** ProSobee base: Control NTP-2000 (*closed squares*), ProSobee (PS) (*closed diamonds*), PS+Whole Casein (*closed circles*), PS+A1-β-Casein (*open triangles*) PS+A2-β-Casein (*open diamonds*)

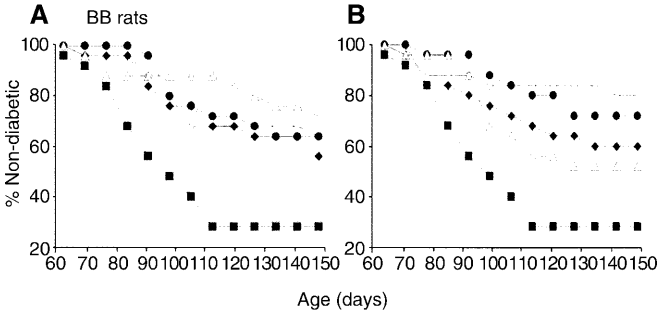

Fig. 2A, B. BB rat survival on various diets. BB rats were fed control, cereal-based NTP-2000 or semipurified diets in which Pregestimil or ProSobee base was supplemented with 10% defined milk fraction. Animals were fed from weaning at 23 days; *n*=26/group. **A** Pregestimil base: Control NTP-2000 (*closed squares*), Pregestimil (PG) (*closed diamonds*), PG+Whole Casein (*closed circles*), PG+A1-β-Casein (*open triangles*), PG+A2-β-Case (*open diamonds*). **B** ProSobee base: Control NTP-2000 (*closed squares*), ProSobee (PS) (*closed diamonds*), PS+Whole Casein (*closed circles*), PS+A1-β-Casein (*open triangles*) PS+A2-β-Casein (*open diamonds*)

Zealand (NOD/NZ) section of the trial (Table 2; Fig. 3). Unfortunately this section had to be abandoned halfway through the study (in terms of numbers of animals put on the experiment) after an outbreak of *Clostridium sp*. that resulted in a large number of deaths. Although the results from this centre are confounded by the infection, the pattern of results up to the time when the infection was detected was similar to that seen at the two other sites.

NOD/Ba mice in the PG group showed lower insulitis values compared to those fed NTP-2000 ($p<0.05$). Other group indices of insulitis in NOD/Ba and BB rats were not significantly different (Fig. 4).

Pancreatic cytokine mRNA content was assessed for groups receiving NTP-2000 or PG-based diets. An effect of diet on cytokine gene expression in pancreas tissue was not observed, although there was a consistent trend toward lower mean IFN-γ and IL-10 concentrations in PG versus animals fed NTP-2000 (Fig. 5). No consistent bias toward Th1 (IFN-γ) or Th2/Th3 cytokines (IL-10, TGF-β) was observed when comparing A1 versus A2 β-casein diets. There

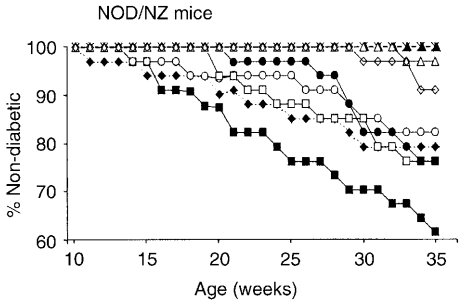

Fig. 3. NOD/NZ mice survival on various diets. NOD/NZ mice were fed control, cereal-based NTP-2000 or semipurified diets in which Pregestimil or ProSobee base was supplemented with 10% defined milk fraction. Animals were fed from weaning to 250 days; initial number *n*=31/group (Note: Infection with *Clostridium sp*. discovered in these animals approximately half way through the study). Control NTP-2000 (*closed squares*), Pregestimil (PG) (*closed diamonds*), PG+Whole Casein (*open circles*), PG+A1-β-Casein (*open squares*), PG+A2-β-Casein (*closed circles*), ProSobee (PS) (*closed triangles*), PS+Whole Casein (*crosses*), PS+A1-β-Casein (*open triangles*), PS+A2-β-Casein (*open diamonds*)

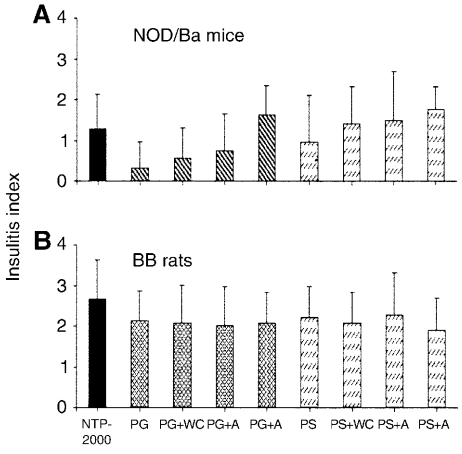

Fig. 4A, B. Insulitis in the pancreas of NOD/Ba mice (**A**) and BB rats (**B**) at conclusion of the study, values are means ± SD

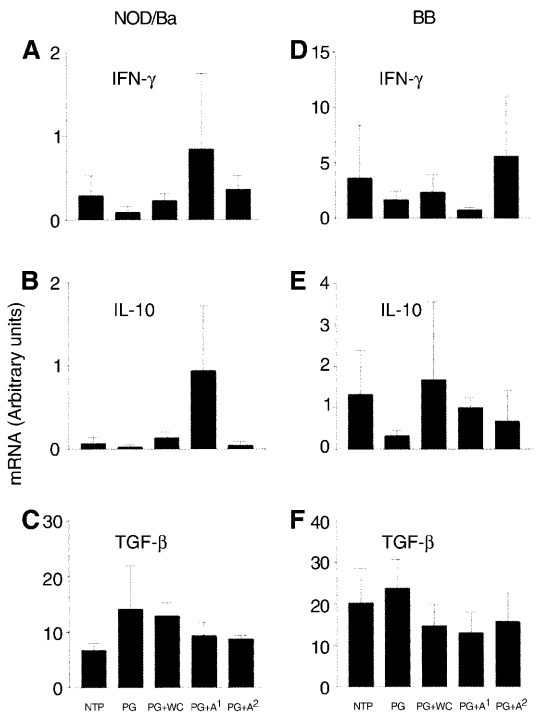

Fig. 5A–F. Cytokine mRNA expression in the pancreas before onset of diabetes. Reverse transcriptase PCR was carried out with total RNA of pancreata of 70–90 day old NOD mice (**A**–**C**) and 79–80 day old BB rats (**D**–**F**) using primers for IFN-γ (**A**, **D**), IL-10 (**B**, **E**), and TGF-β (**C**, **F**). Values are means ± SEM expressed as arbitrary units

was a stronger individual variation of IFN-γ and IL-10 mRNA levels in NOD mice receiving PG+A1 versus PG+A2 , but the opposite was seen in BB rats (Fig. 5).

After being killed body weights of non-diabetic NOD/Ba mice from groups fed with PG and PG+A2 were lower (means ± SD, 25.6±2.6 and 25.2±2.0 g, respectively) than those of control mice (27.6±2.0 g, $p<0.03$). There were no other statistically significant differences in body weights.

Discussion

The finding that the highest diabetes incidence was observed in both NOD mice and BB rats fed the NTP-2000 control diet which is milk-free and largely wheat-based suggests that diet components other than milk could be important in diabetes pathogenesis. Only one of the test diets produced a similar diabetes frequency to NTP-2000, and that was PG+WC fed to NOD/Ba mice; no such effect was evident in NOD/Ba mice fed the PS base. Although still protective, PS+A1 β-casein was more diabetogenic than PS+A2 when fed to BB rats, an effect that was not found in animals fed the PG base diet. The previously reported difference seen in NOD/NZ [13] mice was not confirmed in NOD/Ba mice. The data show that animals fed diets containing A2 β-casein still developed diabetes, an effect not observed in the previous work. The reason for this disparity is not clear but could be due to colony differences [23].

The BB rat experiments showed a higher frequency of diabetes in rats fed PS+A1 compared with those fed PS+A2, in agreement with a previous finding [13]. However, all diets containing A1 or A2 were protective compared with the NTP-2000 control and there was no difference in rats fed the PG base diet. Thus, the A1 fraction was more diabetogenic than A2 only in the presence of the PS diet suggesting an interaction of dietary components. Therefore, the addition of β-caseins to diets had somewhat different effects on diabetes incidence in the two models, but the overall finding was that A1 and A2 β-casein diets were protective and differed little in diabetes-promoting capacity. Pancreatic cytokine mRNA content was not different among groups, nor was the overall degree of insulitis,

except that PG-fed NOD mice had less insulitis than NTP-2000-fed animals, a result that was also reflected in a trend toward less IFN-γ and IL-10 expression.

The worldwide incidence of Type I diabetes has been found to correlate with milk consumption [7, 24], and recent ecological analyses showed an association between the consumption of A^1 β-casein and Type I diabetes in humans [14, 16]. Epidemiological studies highlight associations and do not show cause and effect. It could be possible to differentiate an effect attributable to the presence or absence of an environmental effector but it is extremely difficult to differentiate dose independent abnormal chronic reactions to commonly encountered environmental agents such as dietary antigens. Experimental studies in animals that develop spontaneous diabetes might offer clues that would be difficult to obtain otherwise.

The present study was unique because identical, defined, coded diets were fed to both NOD mice and BB rats in three different locations according to similar protocols. The results strongly suggest that even if milk protein does contribute to the development of diabetes, it is likely that there would still be an appreciable prevalence of the disease in the human population from the consumption of other foods, particularly cereals. This is important as it indicates that dietary interventions involving only the avoidance of milk or changes to milk composition are likely to have a limited influence on the incidence of diabetes in the human population. Dietary exposure to a variety of diabetes-related food components from birth to adolescence could be of critical importance.

Although the trial design did not provide for exact equivalence of nutrients across all diets, within each base diet group, dietary composition was similar. The major differences were in protein composition of the added fractions. It is possible that some other factor or factors that varied among the fractions could be responsible for the different degrees of diabetes incidence. For example, di-(Tyr-Gly) and tri-peptides (Tyr-Gly-Gly) corresponding to the N-terminal fragments of lactalbumin and ß-casein have been shown to have biological activity [25] as do truncated forms of β-casomorphin [14, 26]. Wheat also contains exorphins.

Previous studies on the effect of diet in the BB rat have shown that when fed wheat and soy these animals have a higher incidence of diabetes than when fed hydrolysed casein-based diets [27]. The frequency of diabetes in BB rats fed NTP-2000 in this study is therefore consistent with previous findings, and was similar to the incidence observed in NOD/Ba mice. The low incidence of diabetes in animals fed the hypoallergenic ProSobee diet is also consistent with previous findings where the capacity to promote diabetes was higher in less refined soymeal diets and decreased in more refined infant formulas [27]. The frequency of diabetes was similar and highest in animals fed NTP-2000 in both the Canadian (BB) and UK (NOD/Ba) sections of the trial and is consistent with other reports showing that removal of cows' milk from the diet does not prevent diabetes in NOD mice [28] or BB rats [29]. The results from this study suggest that compared with the control NTP-2000 diet, almost all the other diets inhibited the development of diabetes.

In conclusion, in a multi-centre, international blinded trial, diabetes incidence varied with the feeding of diets from weaning that differed mainly with respect to the source of dietary amino acids. These findings provide strong evidence that diet is an important environmental determinant of Type I diabetes in NOD mice and BB rats regardless of location, different animal quarters, microbial populations and slight variations in animal husbandry. With one exception, NOD/Ba mice fed PG+WC, ProSobee and Pregestimil-based diets were protective and the addition of various milk fractions only marginally altered diabetes incidence, which remained below that of the positive control. The milk-free, mainly wheat-based, NTP-2000 diet consistently produced the highest incidence of diabetes in both BB rats and NOD mice in three widely separated geographic locations. This study showed that the majority of diabetes-prone animals fed whole casein, A^1 or A^2 β-casein diets were protected from developing diabetes. The fact that whole casein was diabetogenic in NOD/Ba mice but protected BB rats from developing diabetes, indicated that animals and humans with distinct diabetes risk haplotypes can react differently to common dietary antigens. These findings show that it is not likely that diabetes could be prevented solely by removing or altering the cows' milk component of the diet and focusing attention on other diabetes promoting foods, particularly wheat.

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