Prophylactic nutritional modification of the incidence of diabetes in autoimmune non-obese diabetic (NOD) mice

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Experiments in rodent models of insulin-dependent diabetes mellitus (IDDM) suggest that destruction of pancreatic β cells can be both initiated and inhibited by certain environmental factors such as dietary constituents. We studied nutritional impact of certain protein sources of natural-ingredient, non-purified (NP) rodent diet on diabetes incidence and insulitis severity in the spontaneous diabetic, non-obese diabetic (NOD) mouse. Long-term *ad lib.* feeding of diets containing wheat flour (800 g/kg), and to a lesser extent soya-bean meal (400 g/kg), were associated with relatively high diabetes incidence (65 and 45% respectively), whereas a diet based on hydrolysed casein (HC; 200 g/kg) as the only source of protein significantly (compared with the wheat-flour diet) inhibited expression of diabetes (22%). Feeding a hypo-allergenic soya-bean-protein hydrolysate resulted in diabetes incidence and insulitis severity similar to that of the soya-bean-meal-fed group. This may indicate that protein hydrolysis *per se* may not be necessary for dietary modification of diabetes in the NOD mouse. The window of vulnerability to diabetogenic diets was found to be between weaning and about 70 d of age. In the diabetic mice insulitis was less frequent in the HC-fed group when compared with those fed NP ($P = 0{\cdot}04$), soya-bean meal ($P = 0{\cdot}03$), soya-bean-protein hydrolysate ($P = 0{\cdot}012$) or wheat flour ($P = 0{\cdot}0002$). In the non-diabetic mice the wheat-flour diet was associated with a high insulitis severity in comparison with the HC group ($P = 0{\cdot}004$). Early avoidance of NP diet was associated with lower degree of insulitis in both diabetic ($P = 0{\cdot}00003$) and non-diabetic mice ($P = 0{\cdot}001$) when compared with the mice fed on the HC diet later in life. These findings are contributing to further clarification of diabetes-promoting dietary constituents, which may have some nutritional implications for IDDM-susceptible children.

Insulin-dependent diabetes: Prophylactic nutrition: Autoimmunity: NOD mice

The destruction of pancreatic islet β cells in insulin-dependent diabetes mellitus (IDDM) in genetically predisposed individuals during the early period of life is currently being attributed to a chronic autoimmune process (Eisenbarth, 1986). A concordance rate for IDDM of less than 50% in identical twins (Barnett *et al.* 1981) indicates that both genetic predisposition and environmental factors are important in the aetiology of the disease. Moreover, in certain ethnic groups the incidence of IDDM reaches those of the host population following migration (Diabetes Epidemiology Research International Group, 1988). These observations together with reports of increasing prevalence of the disease in several countries (Bingley & Gale, 1989) have led to the consensus that IDDM can be both initiated and inhibited by certain environmental factors such as dietary constituents (MacLaren *et al.* 1989).

Studies in the two main spontaneous-diabetic rodent models of IDDM, the bio-breeding (BB) rat and the non-obese diabetic (NOD) mouse, indicate that feeding a hypo-allergenic, hydrolysed lactalbumin or amino acid-based diet can markedly delay and inhibit expression of diabetes (Scott *et al.* 1989*b*). Further studies showed that, contrary to expectations, variation in the amount and source of carbohydrate in a defined diet had a minimal effect on

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diabetes in the BB rat and NOD mouse (Scott *et al.* 1989*b*). Subsequently, several groups pointed to the protein source as the possible modulator of diabetes incidence in these animals (Elliott & Martin, 1984; Scott *et al.* 1985; Elliott *et al.* 1988).

Natural-ingredient, cereal-based, non-purified (NP) rodent diet contains various plant and animal protein sources which are absent in the diabetes-retardant diets (American Institute of Nutrition, 1977). There was a lack of diabetes promotion in the NOD mouse and the BB rat fed on diets containing meat meal, fish meal (Elliott *et al.* 1988; Scott *et al.* 1989*b*) or skim-milk powder (Scott *et al.* 1989*b*; Coleman *et al.* 1990) as the only source of protein. This turned attention to the plant protein sources of NP diet (Coleman *et al.* 1990; Hoorfar *et al.* 1990). However, not enough experimental data are available to clarify the diabetogenic role of plant components from rodent diets in the NOD mouse. Furthermore, the evidence available on crucial time-course for exposure to diabetogenic diets in NOD mouse is contradictory (Elliott *et al.* 1988; Coleman *et al.* 1990).

The NOD mouse spontaneously develops diabetes which in many respects, such as genetic predisposition, involvement of autoimmune process, presence of severe lymphocytic infiltration of the pancreatic islets and metabolic disorders, resembles human IDDM (Leiter *et al.* 1990). Diabetes is observed mainly in female NOD mice (70–80%) with peak incidence at 150–200 d of age, while the incidence for males is less than 30–50%. However, there is a wide incidence of variation in the female gender bias among the breeding colonies worldwide (Leiter *et al.* 1990). This may indicate a role for yet unidentified environmental factors in the aetiology of diabetes, and it, therefore, makes the NOD mouse a unique model for studying exogenous insults. The present study was designed to evaluate the diabetogenic effect of major plant protein (intact or hydrolysed) sources of NP diet, and clarify the crucial time-course of exposure to diabetes-promoting diets.

MATERIALS AND METHODS

Animals

The female NOD/Bom mice were obtained from the breeding colony maintained at Bomholtgaard Breeding Centre (Ry, Denmark). The mice were originated from Shionogi Research Laboratories (Osaka, Japan), and were later transferred to the Diabetes Research Institute at the University of Düsseldorf (Düsseldorf, Germany). From here the Bomholtgaard Centre obtained a breeding nucleus in 1988 which was further inbred by sister–brother mating protocol.

The inbred mice at Bomholtgaard Centre were at the 15th generation of breeding at the time of the present study. The NOD/Bom colony was screened regularly for antibodies against murine viruses: mink virus of mouse, mouse hepatitis virus, pneumonia virus of mouse, Sendai, Ectromelia, Reo-3, as well as mycoplasma. The colony was maintained behind a specific pathogen-free (SPF) barrier, and health monitoring examinations were being done every 3 months. Cyclosporin or other forms of treatment was not applied in the breeding protocol. The NOD/Bom mice breeding colony were fed on autoclaved (15 min at 121°) cereal-based diet (BomChow; Bomholtgaard Centre) and tap water *ad lib.* The incidence of diabetes in the colony was approximately 80% in females, with the age of onset around 150 d, and approximately 40% in males, with the age of onset around 175 d.

For the present study 140 female mice from different litters were weaned at 21 d of age and distributed as evenly as possible (depending on the litter size) across seven diet groups. Groups of five animals were placed in plastic cages containing bedding until 47 d of age and then transferred to stainless steel, wire-bottom cages. The animals were maintained at Bomholtgaard Centre facilities behind the SPF barrier under conditions of uniform temperature ($21 \pm 2°$) and humidity ($50 \pm 5\%$). Lights were on from 06.00 to 18.00 hours.

Table 1. *Composition of various test diets (g/kg)*

Protein source... Ingredient	Hydrolysed casein	Soya-bean meal	Soya-bean-protein hydrolysate	Wheat flour
Protein source	0	400	400	800
Hydrolysed casein	200	0	0	100
Maize starch	300	137	137	0
Sucrose	349·5	350	343	0
Maize oil	50	43	50	40
Cellulose fibre	50	40	40	10
AIN-76 minerals	35	12	12	33·5
AIN-76A vitamins	10	10	10	10
DL-methionine	3	6	6	3
Choline bitartrate	2	2	2	2
L-tryptophan	0·5	0	0	0
L-lysine	0	0	0	0·5
L-threonine	0	0	0	1

Table 2. *Proximate composition of protein sources (g/kg)**

Protein source... Ingredient	Hydrolysed casein	Soya-bean meal	Soya-bean-protein hydrolysate	Wheat flour
Protein	877	510	664	125
Carbohydrate	—	268	175	718
Fat	—	15	5	15
Fibre	—	57	50	3
Ash	83	70	65	9
Water	40	80	41	130

* Values are from suppliers.

The test diets were fed immediately after weaning until 200 d of age. Body weights and food consumptions were measured at 25, 60 and 140 d of age.

Diets

There were seven diet groups with twenty female mice in each group as follows: hydrolysed casein (HC), non-purified cereal-based (NP), soya-bean meal (SM), soya-bean-protein hydrolysate (SH), wheat flour (WF), hydrolysed casein up to 70 d of age and thereafter non-purified diet (HC/NP), and non-purified cereal-based diet up to 70 d of age and thereafter hydrolysed casein (NP/HC). Composition of the test diets is shown in Table 1 and proximate composition of the protein sources is shown in Table 2. With the exception of the NP diet, test diets were modifications of the AIN-76 (American Institute of Nutrition, 1977) formulation with similar levels of energy and protein.

The NP diet was made at Bomholtgaard Centre and contained (g/kg): whole barley 316·5, whole wheat 316·5, bruised soya bean 255·2, fish meal 76·6, vitamin mix 20·4, dicalcium phosphate 8·2, sodium chloride 2·0, methionine 4·6. All components were supplied by DLG (Kjellerup, Denmark). The components were milled gently, mixed for 1 h and formed into pellets under the addition of steam. Micronutrient contents (mg/kg vitamin mix; Kemovit 4035) used in the NP diet were as follows: retinol 2·3, cholecalciferol

0·04, DL-α-tocopherol acetate 100, thiamin 10·00, riboflavin 8·50, nicotinic acid 25, D-pantothenic acid 13·50, pyridoxine 6·0, cyanocobalamin 1·25, choline 500, menadione 2·0, iron 54·29, iodine 0·6, cobalt 1·37, copper 5·04, manganese 62 μg, zinc 23·10 μg, selenium 20 μg.

Ingredients for the test diets were obtained from the following suppliers: hydrolysed casein, enzymic-digested (hydrolysis degree 6·5%), C-0626 (Sigma, St Louis, MO, USA), soya-bean meal and soya-bean-protein hydrolysate (Aarhus Oliefabrik Ltd, Aarhus, Denmark), wheat flour, 85% extracted (Havnemoellerne, Odense, Denmark), maize starch (Apodan, Germany), sucrose (DDS, Copenhagen, Denmark), and AIN-76A vitamins, AIN-76 minerals, DL-methionine, choline bitartrate, L-tryptophan, L-lysine, L-threonine (ICN Biochemicals, Cleveland, OH, USA).

The soya-bean meal was prepared from hexane-extracted, defatted white soya-bean flakes with particle size < 100 mesh. For preparation of soya-bean-protein hydrolysate, white soya-bean flakes were extracted with ethanol (600 ml/l) at 70° in order to produce a protein concentrate, and then exposed to the enzyme (Subtilisin Alcalyse® 2·4L (*EC* 3.4.21.14); Carlsberg, Novo-Nordisk, Bagsvaerd, Denmark) in a ratio of 300:1 for 20 min at 60° and pH 6·6. This step was repeated twice. In order to inactivate the remaining enzymic activity the final product was heated to 90°. Finally, the product was spray-dried with an outlet temperature of 80°. The soya-bean-protein hydrolysate had a hydrolysis degree of 9·76%. This was defined as the percentage of peptide bonds cleaved, by measuring the concentration of primary amino groups (Adler-Nissen, 1979). The anti-trypsin activity was 4·3 units, measured by the method of Smith *et al.* (1980).

Diet ingredients were ground, mixed at room temperature for 1 h in a low-speed, automatic rotating mixer and were vacuum-packed in 1 kg plastic bags, at the Institute of Toxicology, National Food Agency of Denmark. In order to prevent any possible interference from autoclaving, the diets were only irradiated (twice at 4 Mrad; Raychem Ltd, Glostrup, Denmark) before entering the SPF barrier. The diets were stored at 4° while not in use. Fresh feed was filled up in clean feed pots twice weekly. Total food consumption per cage and, thereby, average food consumption per mouse was measured over 1 week. All diets, including the NP, were in powder form and fed *ad lib.* throughout the study.

Diagnosis of diabetes

Mice older than 80 d of age were tested weekly for weight loss and glucosuria with urine tapes (Diabur-Test® 5000; Boehringer-Mannheim, Germany) with arbitrary units: 0, 1+, 2+, 3+, 4+ represented 0, 1, 2·5, 5, 10 or more g/l. Those animals with glucosuria > 2+ and more than 10% weight loss (compared with the previous weeks) were examined for hyperglycaemia by glucose concentration in blood taken from the tail vein at room temperature. Those mice having > 10% weight loss, glucosuria > 2+, and glycaemia > 11·1 mmol/l (Reflolux S®; Boehringer-Mannheim) were diagnosed as having diabetes (by one of us, veterinarian F.D.H.), and were immediately killed (Makino *et al.* 1980). The blood of all non-diabetic animals was similarly examined for hyperglycaemia at the end of the experiment. Non-diabetic mice at the end of the experiment and diabetic mice at diagnosis were anaesthetized with diethyl ether, and blood was collected from the retro-orbital plexus. Age of diabetes onset is here reported as age of mouse at onset of the last glucosuria subsequently confirmed by glycaemia > 11·1 mmol/l (approximately 2 g/l).

Histology

Histological evaluation was performed for both diabetic and non-diabetic mice at kill. Pancreatic tissue was dissected free of connective tissue and fat at necropsy and fixed in Bouin's solution until staining. For staining the pancreatic tissue was embedded in paraffin,

cut into 5 μm sections and stained with haematoxylin and eosin. Using light microscopy the degree of inflammatory infiltration of pancreatic islets (insulitis) was interpreted subjectively by one of us (J.H.) without knowledge of the presence or absence of diabetes. For each mouse three sections were assessed on a scale of 0–4 as follows: 0 morphological unaffected islets, 1 a mild peri-insular infiltration, 2 a more diffuse peri-insular infiltration, 3 a high degree of peri-insular and some intra-insular infiltration, 4 islets entirely dominated by peri- and intra-insular infiltration. All islets at the three pancreatic sections were evaluated and each islet was given a separate score, whereafter each mouse was given an average insulitis score. Finally, an average insulitis grade was calculated for each diet group.

Statistical analysis

Results are expressed as means and standard deviations. Cumulative diabetes incidence was calculated using Kaplan–Meier estimation (this test takes account of both incidence and age of onset). Statistical significance was evaluated by the log-rank test. Student's t test was used for statistical evaluation of insulitis degree. The level of significance (two-sided) was set at $P < 0{\cdot}05$.

RESULTS

Diabetes incidence

Of 137 female NOD mice fed on the seven test diets, sixty-four became diabetic with an average age of onset of 144 (SD 24) d. The diabetes frequency was lowest in the mice fed on diet HC early in life (21 %) and highest in the group fed on diet NP throughout the study (70 %; Fig. 1, Table 3). Among diets containing single protein sources, wheat flour resulted in the highest diabetes incidence (60 %) which was considerably higher ($P = 0{\cdot}011$) than the HC diet (Table 3). Soya-bean meal as the only source of protein was associated with higher incidence of diabetes (45 %) when compared with the HC diet (22 %), but this difference was not significant ($P = 0{\cdot}13$). As far as extraction of soya-bean meal with organic solvents and enzymic hydrolysis is concerned, there was no beneficial effect on the incidence of diabetes in the NOD mice. The time-course study showed that feeding diet NP early in life (21–70 d of age) was associated with significantly ($P = 0{\cdot}008$) higher incidence of diabetes (60 %) when compared with the incidence of diabetes in NOD mice fed on diet NP after 70 d of age (21 %).

Age of diabetes onset

The mean age of onset was highest in the group fed on diet HC throughout the study (162 (SD 4) d), and lowest in group fed on the non-purified diet early in life (126 (SD 20) d). The age of onset was ranked HC (oldest) > soya-bean products > wheat flour > NP (youngest; Table 3). Feeding diet HC slightly delayed the average age of diabetes onset in NOD mice when compared with the NP diet ($P = 0{\cdot}16$; Table 3).

Insulitis grade

All the tested mice had some degree of insulitis. However, in the diabetic mice insulitis was less frequent in the HC-fed group when compared with those fed on NP ($P = 0{\cdot}04$), soya-bean meal ($P = 0{\cdot}03$), soya-bean-protein hydrolysate ($P = 0{\cdot}012$) or wheat flour ($P = 0{\cdot}0002$; Table 4). In the non-diabetic mice variation in the severity of insulitis among the diet groups was less significant, but the wheat-flour diet was associated with a remarkably higher ($P = 0{\cdot}004$) insulitis grade than the HC diet (Table 4). Early avoidance of NP in the HC/NP group was associated with lower degree of insulitis in both diabetic ($P = 0{\cdot}00003$) and non-diabetic mice ($P = 0{\cdot}001$) when compared with the mice fed on HC later in life. The extraction of soya-bean meal with organic solvents followed by hydrolysis had no effect on the insulitis severity when compared with the animals on non-hydrolysed soya-

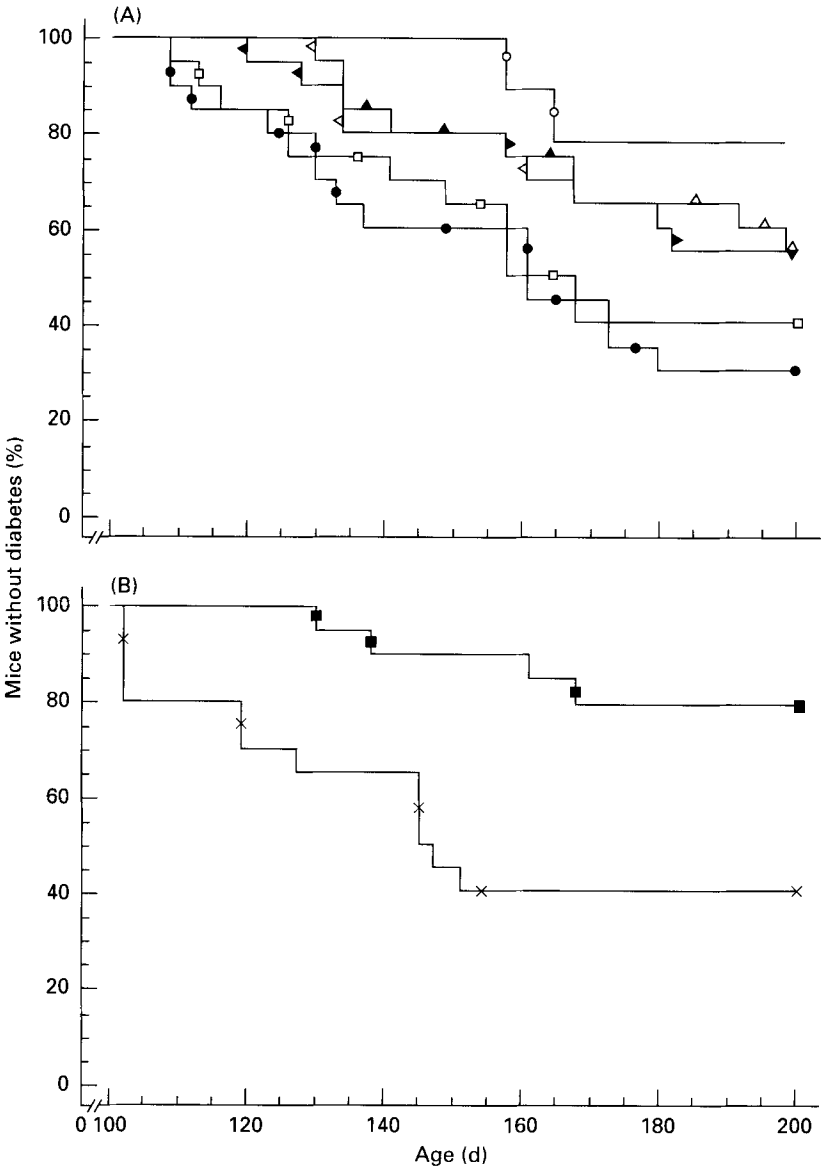

Fig. 1. Survival curves for non-obese diabetic mice fed on diets containing various protein sources. (A) Hydrolysed casein (○), non-purified diet (●), soya-bean meal (△), soya-bean-protein hydrolysate (▲), or wheat flour (□). (B) Hydrolysed casein up to 70 d of age and then non-purified diet (■), or non-purified diet up to 70 d of age and then hydrolysed casein (×). For details of diets and procedures, see Tables 1 and 2 and pp. 599–600.

bean meal. Among the mice tested for insulitis the average grade for the tested diabetic mice (n 63) was significantly higher than the non-diabetics (n 68, $P = 0{\cdot}0006$).

Growth

All mice looked healthy. The mice on diet HC had a slightly lower growth rate at the beginning of the experiment and remained smaller, although not significantly, than the mice in other groups until the end of the experiment (Fig. 2). Food consumption figures

Table 3. *Effect of dietary protein source‡ on diabetes incidence and age of onset in non-obese diabetic mice*

(Mean values and standard deviations)

Diet	*n*	Incidence of diabetes (%)	Statistical significance of difference from HC§: *P*	Onset age (d) Mean	SD	Statistical significance of difference from HC‖: *P*
Hydrolysed casein (HC)	18	22	—	162	4	—
Non-purified (NP)	20	70	0·002	143	25	0·16
Soya-bean meal	20	45	0·13	157	26	0·73
Soya-bean-protein hydrolysate	20	45	0·14	153	23	0·50
Wheat flour	20	60	0·011	141	22	0·087
HC/NP¶	19	21	1	149	18	0·24
NP/HC††	20	60*	0·006	126†	20	0·004

Mean value was significantly different from that of HC/NP group (Kaplan–Meier estimation): *$P = 0·008$.
Mean value was significantly different from that of HC/NP group (Student's *t* test): †$P = 0·058$.
‡ For details of diets see Tables 1 and 2 and pp. 599–600.
§ Calculated using Kaplan–Meier estimation and the log rank test.
‖ Student's *t* test.
¶ Hydrolysed casein to 70 d of age and, thereafter, non-purified diet.
†† Non-purified diet to 70 d of age and, thereafter, hydrolysed casein.

Table 4. *The effect of dietary protein source‡ on subjective scores for severity of insulitis in non-obese diabetic mice*

(Mean values and standard deviations)

	Diabetics				Non-diabetics			
	Mean	SD	*n*	Statistical significance of difference from HC§: *P*	Mean	SD	*n*	Statistical significance of difference from HC§: *P*
Hydrolysed casein (HC)	2·0	0·9	5	—	2·4	0·8	13	—
Non-purified (NP)	3·2	1·1	14	0·04	2·3	0·9	6	0·91
Soya-bean meal	3·1	0·7	9	0·03	3·1	0·7	10	0·04
Soya-bean-protein hydrolysate	3·3	0·6	9	0·012	2·9	0·8	10	0·17
Wheat flour	3·7	0·4	11	0·0002	3·5	0·5	7	0·004
HC/NP‖	3·2	0·8	4	0·09	2·1	0·8	15	0·44
NP/HC¶	3·9*	0·3	11	0·00003	3·7†	0·5	7	0·001

Mean value was significantly different from that of HC/NP group: *$P = 0·05$.
Mean value was significantly different from that of HC/NP group: †$P = 0·0001$.
‡ For details of diets see Tables 1 and 2 and pp. 599–600.
§ Student's *t* test.
‖ Hydrolysed casein to 70 d of age and thereafter non-purified diet.
¶ Non-purified diet to 70 d of age and thereafter hydrolysed casein.

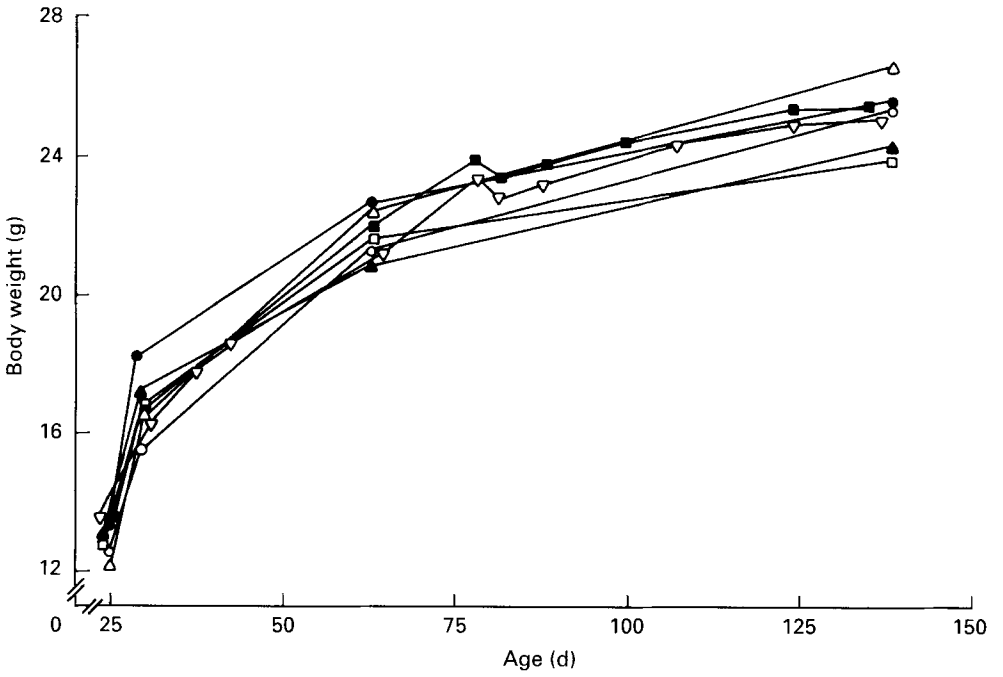

Fig. 2. Mean female body weights throughout the experiment for animals fed on diets containing hydrolysed casein (○), non-purified diet (●), soya-bean meal (△), soya-bean-protein hydrolysate (▲), wheat flour (□), hydrolysed casein up to 70 d of age and then non-purified diet (■), or non-purified diet up to 70 d of age and then hydrolysed casein (▽). For details of diets and procedures, see Tables 1 and 2 and pp. 599–600.

were similar among the diet groups, apart from the group fed on HC in which the consumption was slightly higher in the beginning of the experiment possibly due to the dusty nature of the diet. For the time-course diet groups there was a temporary decrease in the body weights following changing the diets. This was returned to a course similar to that of the other diet groups after 1 week. Despite some variations there were no significant differences in food consumption throughout the course of the experiment (values not shown).

DISCUSSION

There are five major findings in the present study. First, soya-bean materials can, to some extent, be responsible for relatively high incidence of diabetes in NOD mice fed on non-purified diet. Second, extraction with organic solvents and enzymic hydrolysis of soya-bean meal, contrary to expectations, did not modify diabetogenicity of this protein source. Third, wheat flour promoted the expression of diabetes. Fourth, the presence of as much as 100 g HC/kg in the wheat-flour diet did not protect the mice from diabetes. Finally, the crucial time for exposure to diabetes-promoting diets seemed to be between weaning and 70 d of age.

The incidence of diabetes in the HC-fed mice in the present experiment was higher (22%) than that in female NOD mice fed on a similar diet during the first 40 weeks of age in two other laboratories (2·1% Elliott *et al.* 1988, 0% Coleman *et al.* 1990). In addition to possible variations in the breeding protocol and the role of viral infections (Wilberz *et al.* 1991), this could be due to the variation in the sources of hydrolysed casein. While hydrolysed casein in the present experiment was supplied separately and mixed with the

other ingredients in the laboratory, the aforementioned workers fed an HC-based infant formula to their mice. The possible differences in the degree of hydrolysis and/or purity of other ingredients could account for the difference in the incidence.

Elliott *et al.* (1984, 1988) concluded that the protective effects elicited by an amino acid-based diet and the infant formula Pregestimil may be attributable to lack of intact proteins in these diets. However, the lack of effect of protein hydrolysis on diabetogenicity of soya-bean meal in the present study may suggest that intact proteins *per se* may not be necessary for full expression of diabetes. Furthermore, animal protein sources such as fish meal (Elliott *et al.* 1988) and skim-milk powder (Coleman *et al.* 1990), despite containing intact proteins, failed to produce a high incidence of diabetes in NOD mice, whereas the following plant protein sources had some degree of diabetes-promoting effect: brewer's yeast resulted in a 50% incidence (Coleman *et al.* 1990), soya-bean meal and wheat flour in the present study resulted in 45 and 60% respectively.

Since wheat products are a major protein source in non-purified diets, their diabetogenic role in both the BB rat (Scott *et al.* 1989*b*) and the NOD mouse has been under special focus. Reports of coexistence of coeliac disease and IDDM in newly diagnosed diabetics (Thain *et al.* 1974; Chamber, 1975), which both associate with HLA markers DR3 and DQ (Koivisto *et al.* 1977), and the well-studied role of the gliadin fraction of gluten in the induction of coeliac disease led to speculation on a possible diabetes-inducing role of wheat gluten (Scott *et al.* 1989*b*).

While Elliott *et al.* (1988) and Coleman *et al.* (1990) reported no diabetogenic effect (4·6 and 18·1% incidence respectively) of feeding up to 100g gluten/kg added to an HC- or casein-based diet, we found wheat flour with as little as 120 g protein/kg produced diabetes in 60% of animals in the present study. However, the effect of feeding pure gliadin as the only source of protein in a well-controlled study remains to be elucidated, as wheat flour also contains constituents other than gluten. Nevertheless, it would be difficult to suggest gliadin as the only promoter of diabetes as the present study showed materials containing no gliadin, e.g. soya-bean meal, to be diabetogenic to a similar extent.

As in the NOD mice, soya-bean meal was also found to be diabetogenic in the BB rat (Brogren *et al.* 1989). This effect was modified in the BB rat when the soya-bean meal was extracted with organic solvents and subsequently underwent enzymic hydrolysis (Brogren *et al.* 1989). Coleman *et al.* (1990) also reported inhibition in the expression of diabetes in the NOD mice fed on a chloroform–methanol-extracted natural-ingredient diet. However, in the present study we found no beneficial effect of feeding ethanol-extracted, soya-bean-protein hydrolysate to NOD mice. Here it should be emphasized that we extracted a single component of the diet, whereas Coleman *et al.* (1990) extracted the entire diet. This aspect needs more investigation with other types of soya-bean-protein hydrolysates prepared differently. As discussed by Hoorfar *et al.* (1991), other materials such as various metabolites of plant origin present in non-purified diets may exert a synergistic effect with intact proteins.

The present study found some degree of insulitis to be universal in the NOD mice tested, while others (Elliott *et al.* 1988; Coleman *et al.* 1990) have reported inhibition of insulitis in a majority of NOD mice fed on an HC-based infant formula. Nevertheless, subsequent studies by the latter group revealed a much higher degree of insulitis in pancreases sampled from Pregestimil-fed mice at 8 and 12 weeks of age (E. H. Leiter, personal communication). These variations might be due to some uncontrolled environmental variable. It is not clear whether the type of infiltrating cells seen in NOD mice on the HC diet was identical to those in mice fed on the NP diet. Also, the time-course and severity of insulitis on low-diabetogenic diets in NOD mice need to be studied.

In conclusion, diet can not only modify the incidence of diabetes and age of onset, but

to a lesser extent the degree of insulitis as well. The question of how diet acts to unmask and trigger genetic predisposition to develop insulin-dependent diabetes in animal models of IDDM is complex. Although the immune system is thought to play a major role in destruction of β cells, preliminary efforts to show any significant changes in the humoral and cellular immunity in, for example, the BB rat following feeding beneficial diets have failed (Scott *et al.* 1989*a*, 1990). The interaction of nutrition and the immune function of the NOD mouse before onset of diabetes needs to be studied. In addition, the suggestion that the nutritional factors affect the functional state of β cells and, thereby, increase antigen expression and/or susceptibility to the toxicity of diabetogenic agents should be considered (Buschard, 1991).

The progress in immunological methods has facilitated clinical prediction of diabetes in high-risk relatives of IDDM subjects. This would hopefully aid investigators in early screening for susceptible subjects, which may then pave the way for nutritional studies in predisposed children.

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