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Dietary prevention of diabetes in the non-obese diabetic mouse

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Summary. Diabetes prone NOD female mice were fed diets containing different proteins from just before weaning. Only mice receiving meat meal or casein as the protein source developed diabetes at the rate expected from this colony. Lactalbumin and gluten did not precipitate diabetes except in a small number. Casein hydrolysate in lieu of protein protects against overt diabetes, but only if introduced early. The animals which did not show overt diabetes nevertheless had intermittent trace glycosuria and the majority showed mild degrees of periinsular lymphocytic infiltration.

Key words: NOD mouse, diabetes, diet, casein.

The inbred Japanese derived albino mouse, the females of which develop insulin-dependent diabetes without obesity (NOD) has been previously shown to respond to nicotinamide as both a preventive measure, and to a limited extent, as a treatment of diabetes [1]. We have previously shown that a protein-free diet introduced before weaning to the insulin dependent prone BB rat prevents diabetes [2]. The purpose of this study was to establish whether a protein-free diet, sufficient in amino acids could similarly prevent diabetes in this diabetes prone strain of mouse.

Materials and methods

NOD mice were obtained from a strain held at the Ehime University, Japan. About 25-30% of the females develop diabetes between 120 and 200 days of age when fed laboratory chow in Japan, 30% developed diabetes in our own laboratory (see also below).

All of the female animals used in these experiments were derived from two breeding pairs, the dams of which both developed overt diabetes after litter production. However, no litters were admitted to the experiments if their dams were overtly diabetic.

Litters were randomly allocated to 8 different dietary regimes (Table 1) at 12 days of age. The litters were weaned at 21 days of age and the females separated at 30 days of age, 3-5 per cage. There was no male/female ratio difference in any of the groups. Urine was tested for glucose (Testape) three times a week and the animals were weighed at these times from 120 days of age. The diets and urine testing were continued to 250 days. If more than trace glycosuria occurred, the animals were tested daily, and designated diabetic if heavy glycosuria persisted for more than three consecutive days. The others were diagnosed non-diabetic, although all showed intermittent but not persistent trace glycosuria, and their islets were often abnormal (vida infra). After these three days the animals were killed and diabetes confirmed by estimating blood glucose. The pancreata were processed for histology after Bouin's fixation, sectioned and stained with haematoxylin and eosin. All "non-diabetic" animals were killed at 250 days and their blood similarly examined. About

Table 1. Dietary Regimes

	Number of litters	
1. Base[a]	10	Pregestimil powder formed by pressure into cakes
2. Base and glycine	5	2% W/W glycine in Pregestimil
3. Base and gluten	10	2% W/W commercial wheat gluten in Pregestimil
4. Base and lactalbumin	10	2% W/W commercial cow's milk lactalbumin in Pregestimil
5. Base and casein	10	2% W/W commercial lactic casein in Pregestimil
6. Chow[b]	10	Commercial mouse chow
7. Base and casein	5	10% W/W commercial lactic acid casein and Pregestimil
8. Chow and base (late)	5	Pregestimil powder introduced at 90 days of age, after being fed chow from weaning

[a] Pregestimil (Mead Johnson Co., Evansville, Indiana, USA) is an allergenic infant formula, based in enzymically hydrolysed cow's milk casein, further treated with charcoal to remove antigenic peptides. It contains 12.8 g protein equivalent/100 g powder, 18.3 g fat as corn oil and coconut oil and 62 g carbohydrate as corn syrup and modified tapioca starch. It contains minerals and vitamins to at least conform with Codex Alimentarius standards for infant formulae. It is supplemented with cystine, tyrosine, tryptophan and taurine. [b] Diet 86 New Zealand Stockfoods, contains wheat - 47.5%, barley - 25%, meat and bone meal - 15.5%, grass meal - 5%, lime - 2%, yeast - 5%, minerals and salts added. Meat and bone meal was derived from cows, pigs and sheep and includes offal.

half of the "non-diabetic" animals' pancreata were examined histologically and their results only included if four or more islets were seen. Since the presence of insulitis was often patchy the histological changes were graded "blind" on a scale of 4 depending on the severity and uniformity of lymphocytic infiltrates.

Results

All diabetic animals showed random blood glucose levels of >13 mmol/l and none of the non-diabetic animals showed levels that high. Histological examination of the diabetic pancreata showed extensive insulitis and B-cell loss as described by Makino et al. [3]. Some non-diabetic animals showed extensive insulitis and some B-cell loss, but many islets showed an apparently normal complement of B cells and no insulitis. The prevalence and severity of histological changes parallel the incidence of diabetes in the groups in which it was measured (Fig. 1). The rate of clinically manifest diabetes and the age at which it appeared in the various dietary groups is shown in Table 2. Weights of the animals prior to development of diabetes were similar in all groups.

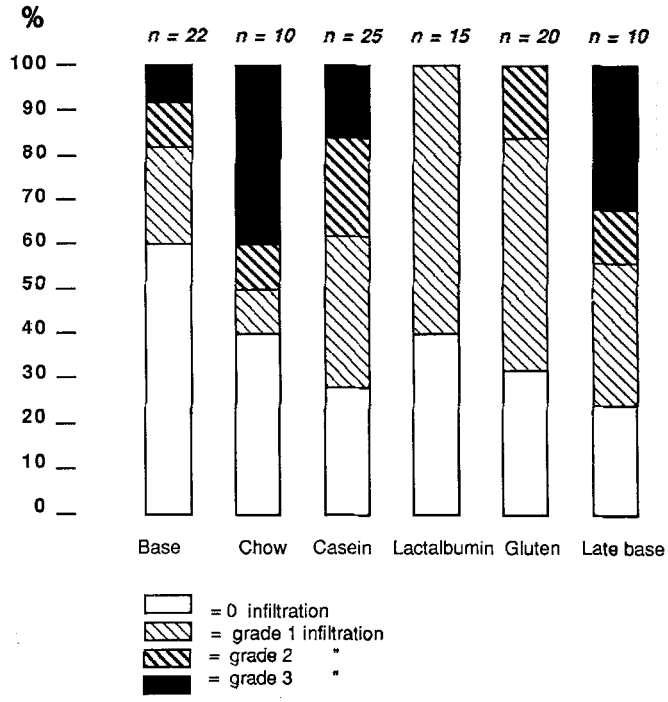

Fig. 1. Degree of insulitis in pancreata of non-diabetic mice without overt diabetes at 250 days

Discussion

As with the BB rat [2] a non-protein diet (in this case Pregestimil or Pregestimil + 2% glycine) largely prevents diabetes in these genetically susceptible mice. The addition of 2% lactalbumin or 2% gluten did not alter this effect. The late introduction of the Pregestimil after chow feeding did not affect the diabetes rate when compared with chow introduced early. It appears that intact protein per se (such as gluten or lactalbumin) is not necessarily an environmental trigger for the development of diabetes, but some unknown substance in chow, or more specifically commercial casein, can be such a trigger. The way in which casein precipitates the diabetes might be chemical (e.g. generation of diabetogens such as nitrosamines), or immunological. The experiments reported above were not designed to answer these possibilities.

It is an interesting speculation that ethnic groups who traditionally introduce cow's milk into the diets of infants at an early stage have a high childhood diabetes rate, whereas those groups who suckle their infants for relatively long periods of time, and do not have ready access to cow's milk, have a very low rate. This speculation is in part supported by the findings of Borch-Johnsen et al. [4] who found childhood diabetic patients were fed for a shorter time on human milk than their non-diabetic siblings or peers. The study was done in a traditional dairy country, but the variable of partial cow's milk feeding or type of weaning diet was not recorded. Scott et al. [5] also found that dietary modification affects the rate of diabetes in the BB rat, though it is uncertain which dietary constituent(s) was involved.

Table 2. Incidence of diabetes in dietary groups

	Dietary groups	Number of animals non-diabetic at 250 days	Number diabetic and percentage (%)	Age at diagnosis (days)
Base	1	48	1 (2.1)	194
Glycine	2	21	1 (4.6)	247
Gluten	3	42	2 (4.6)	174, 183
Lactalbumin	4	40	1 (2.4)	222
Casein	5	41	7 (14.6)	158, 167, 171, 187, 187, 201, 247
Chow	6	38	14 (26.9)	130, 135, 140, 150, 152, 157, 177, 178, 200, 203, 205, 250, 220, 223
Casein 10%	7	17	4 (19.1)	153, 159, 170, 210
Chow late base	8	19	6 (24)	150, 170, 187, 200, 210, 240

Statistical note: Gp 5 vs Gp 1, 2, 3, 4 $p=0.008$[a]; Gp 6 vs Gp 1, 2, 3, 4 $p<0.001$[a]; Gp 7 vs Gp 1, 2, 3, 4 $p=0.013$[a]; Gp 8 vs Gp 1, 2, 3, 4 $p=0.001$[a].

[a] Fisher's exact test

The animals receiving chow showed a rate of diabetes similar to that reported for this colony in Ehime. The animals given 10% casein did not show a greater

adverse effect than 2% casein. The late introduction of the protein-free diet did not prevent diabetes.

We conclude that both cow's milk casein and some unidentified substance in commercial mouse chow appear to be dietary triggers of diabetes in the non-obese diabetic mouse, when introduced at weaning. This may have implications for the human disease.

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References

1. Yamada K, Nonaka K, Hanafusa T, Miyazaki A, Toyoshima H, Tarui S (1982) Preventive and therapeutic effects of large-dose nicotinamide injections on diabetes associated with insulitis. An observation in non-obese diabetic (NOD) mice. Diabetes 31: 749–753
2. Elliott RB, Martin JM (1984) Dietary protein: a trigger of insulin-dependent diabetes in the BB rat? Diabetologia 26: 297–299
3. Makino S, Kunimoto K, Muraoka Y, Mizushima Y, Katagiri K, Tochino Y (1980) Breeding of a non-obese, diabetic strain of mice. Exp Anim 29: 1–13
4. Borch-Johnsen K, Mandrup-Poulsen T, Zachau-Christiansen B, Joner G, Christy M, Kastrup K, Nerup J (1984) Relation between breast-feeding and incidence rates of insulin-dependent diabetes mellitus. Lancet II: 1083–1086
5. Scott FW, Mongeau R, Kardish M, Hatina G, Trick KD, Wojcinski Z (1985) Diet can prevent diabetes in the BB rat. Diabetes 34: 1059–1062

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