Int J Diabetes & Metabolism (2009) 17:37- 40

A low carbohydrate, high fiber diet containing a processed maize by-product increases the incidence and advances the onset of diabetes in NOD mice

Robert B Elliott, Nicola J Beckman, Olivia D Andersen, Marija Muzina, Stephen JM Skinner
Living Cell Technologies NZ Ltd, Manukau, New Zealand

Abstract
Type I diabetes is reported to be caused by a combination of genetic predisposition, diet and immune exposure. Small animal models, such as the Non-obese diabetic (NOD) mouse, are often used to test potential therapies that may prevent, delay or cure the disease. This model of Type 1 diabetes may require large numbers of mice to demonstrate the effects of intervention to prevent or treat the disease and its sequelae. Our aim was to find a non-fat dietary component that would increase the incidence of Type 1 diabetes in the NOD mouse and allow smaller but statistically valid experiments. We have developed a relatively well-balanced diet that advances the mean age of onset of Type 1 diabetes from 209 days to 175 days and increases the incidence of Type 1 diabetes from approximately 50% to 76%. This diabetogenic diet was fed from weaning and contains a high proportion (58.5%) of a processed maize by-product high in fibre and protein but with low carbohydrate. Normal ground maize was not diabetogenic. Advanced glycosylation end-products, known to be toxic to pancreatic beta-cells, were measured and were higher in the diabetogenic processed maize diet than in the control diet. Our results suggest that this processed maize by-product, used principally as a farm animal feed throughout the developed world, promotes diabetes in the NOD mouse and is possibly caused by advanced glycosylation end-products.

Key Words: *Type I diabetes, NOD mouse, diet, glucose, insulin, processed maize, advanced glycosylation end-products*

Introduction

Type I diabetes is a major health problem and economic cost to most developed and developing economies.[1,2] It is also of great social cost, usually requiring massive maternal and family commitment to the care of sick children.[3] The causes are reported to be a combination of genetic predisposition, diet and immune exposure.[4,5] Small animal models are often used to test potential therapies that may prevent, delay or cure the disease.[6,7] During the investigation of an inbred genetically predisposed Type I diabetes mouse model (female NOD LtJ and NOD HT strains), it was found that the rate of diabetes was low, requiring a relatively large number of mice in each group to obtain sufficient statistical power to provide useful data. Several different diets have been reported to increase the susceptibility of NOD mice to diabetes.[8,9,10,11] In order to increase the Type I diabetes rate and lower the numbers of mice required per group, a number of dietary combinations were tested that might increase Type I diabetes. A commercial processed maize by-product containing relatively high concentrations of advanced glycosylation end-products was investigated and found to increase the rate of diabetes in these genetically predisposed mouse strains.

Materials and Methods

Animal Studies

Ethical approval for these experiments was granted by the Animal Ethics Committee of Living Cell Technologies Ltd under the guidelines approved by the National Animal Ethical Committee of New Zealand. Mice were obtained from breeding colonies of the Animal Resources Unit, University of Otago, New Zealand. Breeding was carried out to maintain the rate of diabetes by crossing the progeny of diabetic mice. Only female mice were selected for experiments. They were separated from the dams at weaning, males were culled and the females received either standard chow (Teklad 2018, Harlan Teklad, Oxon, UK) as the Control Diet (CD) or the experimental diet (Diabetogenic Diet, DD). They were allowed ad lib access to water and chow. From 80 days of age the urine was tested each week for glucose with Diastix (Bayer, Dublin, Eire). When a urine sample was positive for glucose the mice were subsequently confirmed diabetic if blood glucose, determined by Super Glucocard II (Arkray, Kyoto, Japan) on a blood drop sampled from the tail, was >12mM. Mice were determined to be disease-free if they survived for 250 days without elevated blood glucose.

Diet Preparations

The experimental diabetogenic diet (DD, Table 1) was formulated based on a combination of skim milk powder (Fonterra, NZ), tapioca starch (Penford, NSW, Australia), safflower oil (Tasti, Auckland, NZ) and coconut oil (Oilseed Products, Auckland, NZ) with mineral and vitamin supplements (Harlan Teklad, Oxon, UK). A commercial processed maize by-product remaining after oil and starch extraction, finely ground and sieved “maize expeller cake” (Avon Feed, Penford, Auckland, NZ), was used as the major

Received on: 01/04/2009
Accepted on: 06/06/2009

Correspondence to: SJM Skinner, Living Cell Technologies NZ Ltd, Manukau 2025, New Zealand, Email:sskinner@lctglobal.com

protein and fiber component of DD. Typically the experimental DD pellets (2kg per batch) were made by first mixing the milk powder (400g) with cold water (1000ml). The Avon Feed (1170g), tapioca starch (200g), vitamin mix (15g) and mineral mix (15g) were then added and mixed until homogeneous. The coconut oil (100g) and safflower oil (100ml) were then added and mixed until homogeneous. The mixture was formed into pellets (approximately 40cc each) and dried for 15-18h at 60oC (residual moisture was 5-7%). In some studies unprocessed maize (PCL, Auckland, NZ) was ground in a domestic blender and used at a weight equivalent to Avon Feed in diet formulation.

Analytical Methods

The Control and Diabetogenic Diets were analyzed for Advanced Glycosylation End-products (Advanced Maillard Products) using the FAST Index method.[12,13]

Statistics

The differences between the groups were determined by T-test for unequal variance and deemed significant if $p<0.05$.

Results

The incidence of Type 1 diabetes in susceptible mouse strains (NOD LtJ and NOD HT) was maintained by careful breeding procedures, crossing male and female diabetic mice from the same colony. In preliminary studies on a NOD mouse strain with a low incidence of diabetes, a diet containing 20% of finely ground maize expeller cake (Avon Feed) resulted in a diabetes incidence of 6/23 (26%). The control group for this study was fed a diet containing 20% of unprocessed ground maize and had an incidence of 2/26 (8%). The onset of diabetes was earlier (176 days age) in the 20% Avon Feed group than in the 20% unprocessed ground maize group (217.5 days age, $p< 0.05$). This result indicated that the processed maize expeller cake might be used to maximize the diabetes incidence.

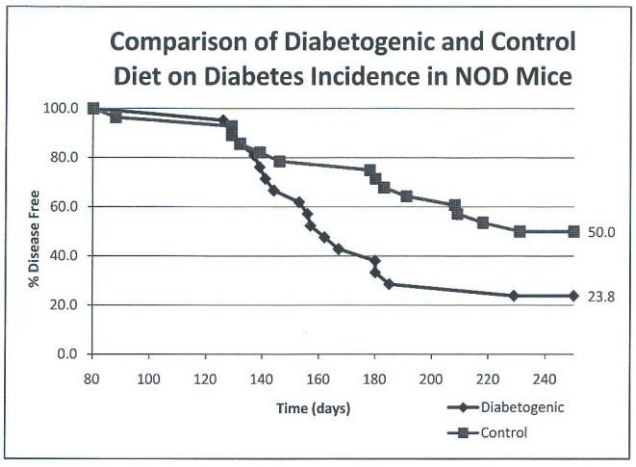

Figure 1: The disease state was defined as blood glucose >12mM and was limited to the period 80 to 250 days of age. The mean age of onset in each group was compared by two-tailed t-test. The results (mean +/- standard deviation) were CD 209 +/- 50.6 and DD 179+/- 46.6 ($p< 0.04$). Most of the diabetes in mice fed DD occurred before 180 days whereas about half of those on CD became diabetic after 180 days.

Table 1: Avon Feed is a hot acid-processed ground maize expeller cake marketed as a feed for farmed animals. Vitamin and mineral mixes are designed for rodent diets. All other components are sold as human foods and purchased from wholesale suppliers.

Ingredients	**%**
Avon Feed	58.5
Skim milk powder	20.0
Tapioca starch	10.0
Coconut oil	5.0
Safflower oil	5.0
Vitamin mix	0.75
Mineral Mix	0.75
Moisture (after completion)	(7.0)

Table 2: Comparison Diabetogenic Diet and Control Diet

Nutrients and Ingredients in DD (%)	Diabetogenic Diet (DD)	Control Diet (CD)
Total Protein	18.02	18.90
Avon Feed	10.50	
Skim Milk Powder	7.20	
Tapioca Starch	0.32	
Total Oil	11.0	6.0
Coconut Oil	5.0	
Safflower Oil	5.0	
Corn Oil (residue in Avon Feed)	1.0 (approximate)	
Total Carbohydrate	19.1	57.33
Tapioca Starch	9.7	
Avon Feed	9.4	
Total Fiber	28.6	3.8
Soluble fiber	6.7	
Hull fiber	21.9	
Sugar	11.0	4.93
Skim milk powder (lactose)	11.0	

In the second experiment, a strain of high incidence Type I diabetes-prone NOD female mice were fed the Control Diet (CD), a standard rodent chow (Teklad 2018), from weaning. They were found to have Type I diabetes incidence of 50% from 80 to 250 days of age. When the same genetic stock of female NOD mice were fed a diet containing 58.5% of the processed maize by-product (Diabetogenic Diet, DD, Table 1) the diabetes incidence was significantly increased from 14/28 (50%) with CD to 16/21 (76%) of those mice fed on DD ($p<0.05$). The onset of Type I diabetes was earlier in the mice fed the DD (Figure). The mean age for diabetes onset in the mice fed DD was significantly earlier at 179 days than in mice fed CD, where the mean age for onset was 209 days ($p<0.05$).

In comparing the dietary components of DD and CD, there is no obvious indication that diabetes should occur earlier or with greater incidence (Table 2). The CD (Teklad 2018) is

far higher in carbohydrate (57%) than DD (9.8%), a characteristic that would not predict diabetogenic activity. The fiber content of DD is ten times that of CD while oil in DD is almost double that in CD.

Analysis of the two diets for indications of component modification during processing using the FAST Index method[12,13] showed a mean index of 21.65 in the Control Diet compared to a mean index of 37.01 in the Diabetogenic Diet.

Discussion

The principle finding in these experiments is a useful diet for increasing the rate of diabetes in a common animal model for Type 1 diabetes, the NOD mouse. Diabetes in the NOD mouse occurs only in females to any significance. In males it is usually <2%. It is reported that the NOD in its various strains (LtJ, HT) has a genetic predisposition to diabetes but the incidence caused by genetic predisposition can be strongly influenced by breeding, diet and by environmental factors.[8,9,14] It is therefore a useful model in that it reflects many of the genetic and environmental facets of human Type I diabetes. The NOD mouse does not, however, model human Type 1 diabetes accurately in that few males become diabetic and the onset is after reproductive maturity.

In some of our early work we found the Type 1 diabetes rate in NOD mice was so low (8% to 20% diabetic) that relatively large numbers of mice were required to give sufficient statistical power for determining whether a therapeutic intervention was statistically significant.[14] With the observation that a 20% Avon Feed component in the diet was diabetogenic, we attempted to maximize the effect by raising the Avon Feed content to 58.5%. The protein content was matched to the control diet by adding 20% skim milk powder. The protein contents of the two diets used in this experiment (approximately 18 and 19%, Table 2) are nutritionally sufficient but below levels that might influence diabetes.[10] Carbohydrate is low and oil relatively high in DD compared to CD (Table). However the oil content of DD would rank as equivalent to, or lower than, a normal diet in most studies.[11]

Mice fed DD gained weight from weaning throughout the study in a manner comparable to CD. There were no obvious changes seen in alertness and activity between CD and DD groups during development. There was a similar increase in mouse running and spontaneous activity observed in the week preceding high urinary and blood glucose in both groups.

The maize by-product Avon Feed is produced when maize is processed to extract oil and cornstarch. The remaining material is typically protein (19%) and fiber (68%) with 10.5% moisture and traces of corn oil and starch (<1.2% each). During the processing the mixture is acidified with sulfurous acid (pH 3-5 and with chemical reducing activity) and heated to 50^{o}C for 36-100h. There is a strong possibility that the processing may add to, or alter, components under these conditions, as may often occur in food processing.[15,16] The by-product in many modifications is widely used for farm animal feed in regions where corn oil and starch are produced throughout the developed world. When fed to ruminants or poultry it is likely that the substances that may be diabetogenic in mice may have no noticeable effect. It is quite possible that ruminant or gut bacteria in most animals may deactivate the potentially diabetogenic substances. Alternatively, the inbred NOD strains of female mice may be serendipitously selected for genetic sensitivity to these substances.

Our review of the scientific and commercial databases has not shown whether people are exposed to this processed maize material as a dietary substance. It may be superficially attractive as a low calorie food source for human nutrition since it is principally protein and fiber. Soy and flax cakes have been tested and found suitable for human consumption.[18] It is also possible that other cereals treated with heat and chemical agents may contain significant amounts of diabetogenic materials such as advanced glycosylation end-products.[19]

Acknowledgement

This study was supported by the Child Health Research Foundation of New Zealand and Living Cell technologies NZ Ltd.

References

1. Disdier-Flores OM, Rodríguez-Lugo LA, Pérez-Perdomo R, Pérez-Cardona CM. The public health burden of diabetes: a comprehensive review. P R Health Sci J 2001; 20:123-130.
2. Bjork S, Kapur A, King H, Nair J, Ramachandran A. Global policy: aspects of diabetes in India. Health Policy2003; 66:61-72.
3. Sullivan-Bolyai S, Deatrick J, Gruppuso P, Tamborlane W, Grey M. Constant vigilance: mothers' work parenting young children with type 1 diabetes. J Pediatr Nurs 2003; 18:21-29.
4. Vlassara H, Striker G. Glycotoxins in the diet promote diabetes and diabetic complications. Curr Diab Rep 2007; 7:235-241.
5. Scott FW, Marliss EB. Conference summary: Diet as an environmental factor in development of insulin-dependent diabetes mellitus. Can J Physiol Pharmacol 1991 69:311-319.
6. Leiter EH, Coleman DL, Eisenstein AB, Strack I. Dietary control of pathogenesis in C57BL/KsJ db/db diabetes mice. Metabolism 1981; 30:554-562.
7. Elliott RB, Reddy SN, Bibby NJ, Kida K. Dietary prevention of diabetes in the non-obese diabetic mouse. Diabetologia 1988; 31:62-64.
8. Hoorfar J, Buschard K, Dagnaes-Hansen F. Prophylactic nutritional modification of the incidence of diabetes in autoimmune non-obese diabetic (NOD) mice. Br J Nutr 1993; 69: 597-607.
9. Flohé SB, Wasmuth HE, Kerad JB, Beales PE, et al. A wheat-based, diabetes-promoting diet induces a Th1-type cytokine bias in the gut of NOD mice. Cytokine 2003; 21:149-154.

10. Schneider K, Laube H, Linn T. A diet enriched in protein accelerates diabetes manifestation in NOD mice. Acta Diabetol. 1996; 33: 236-240.
11. Linn T, Strate C, Schneider K. Diet promotes beta-cell loss by apoptosis in prediabetic nonobese diabetic mice. Endocrinology 1999; 140: 3767-3773.
12. Leclère J, Birlouez-Aragon I. The fluorescence of advanced Maillard products is a good indicator of lysine damage during the Maillard reaction. J Agric Food Chem. 2001; 49:4682-4687.
13. Birlouez-Aragon I, Leclere J, Quedraogo CL, Birlouez E, Grongnet JF. The FAST method, a rapid approach of the nutritional quality of heat-treated foods. Nahrung. 2001; 45:201-205.
14. Reddy S, Bibby NJ, Wu D, Swinney C, et al. A combined casein-free-nicotinamide diet prevents diabetes in the NOD mouse with minimum insulitis. Diabetes Res Clin Pract. 1995; 29:83-92.
15. Elliott RB. Diabetes -- a man-made disease. Med Hypotheses. 2006; 67; 388-391.
16. Sandu O, Song K, Cai W, Zheng F, Uribarri J, Vlassara H. Insulin resistance and type 2 diabetes in high-fat-fed mice are linked to high glycotoxin intake. Diabetes. 2005; 54:2314-2319.
17. Liu G, Hughes CL, Mathur R, Foster WG, Davis VL, Magoffin DA. Metabolic effects of dietary lactose in adult female rats. Reprod Nutr Dev 2003; 43:567-576.
18. Mitra A, Bhattacharya D. Oil Cakes for Human Consumption in NIDDM. Indian Journal for the Practising Doctor 2007; Volume 4 (ePub 2007-03 - 2007-04)
19. Sebeková K, Somoza V. Dietary advanced glycation endproducts (AGEs) and their health effects-PRO. Mol Nutr Food Res 2007; 51:1079-1084.