Modified Arabinoxylan from Rice Bran (BioBran/MGN-3) Improves Glucose Tolerance in NIDDM Adult Rats Given Streptozotocin as Neonates

Ikuo OHARA and Kumiko ONAI
Faculty of Home Economics, Aichi Gakusen University, Aichi, Japan
Hiroaki MAEDA
Daiwa Pharmaceutical Co., Ltd., Tokyo, Japan

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Abstract

The effect of BioBran/MGN-3 on glucose tolerance was studied in adult non-insulin dependent diabetes mellitus (NIDDM) rats that had been caused by intraperitoneal injection of 100 mg per kg streptozotocin (STZ) at 1.5 days of age.

Following weaning, the animals were divided into 3 groups, 5 control rats fed a 1.7% cellulose diet, 7 diabetic rats fed a 1.7% cellulose diet and 8 diabetic rats fed a 1% BioBran/MGN-3 (+0.7% cellulose) diet. Rats had free access to one of experimental diets and water for 60 days. Oral glucose tolerance tests were performed at 8 weeks of age. Trunk blood was collected and plasma levels of insulin, glucose, triglycerides, total cholesterol, HDL-cholesterol, total protein, albumin and urea nitrogen were measured.

Plasma glucose levels in diabetic rats fed the 1.7% cellulose diet increased significantly faster and to

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higher levels than those of the normal control in an oral glucose tolerance test. In the NIDDM rats fed the BioBran/MGN-3 diets, the rapid rise of plasma glucose levels was depressed. Among the various other biochemical measurements only the plasma total cholesterol levels were significantly reduced by the administration of BioBran/MGN-3.

BioBran/MGN-3 holds promise as a dietary fiber supplement for the treatment of diabetes.

Introduction

Dietary fiber, based on its physicochemical nature, is known to have preventive actions in various disorders (Tsuji 1988). For example, there have been many reports on the curative and preventive effects of dietary fiber on diabetes mellitus (Anderson et al. 1991; Goswamy and Mani 1985; Morgan et al. 1990). Water-soluble dietary fiber lowers plasma cholesterol and maintains blood glucose concentrations within a suitable range (Guévin et al. 1996; Yuan et al. 1998; Groop et al. 1993; Sugano et al. 1988; Aoe et al. 1988). Mechanisms underlying these effects are not fully understood. The delay of gastric emptying (Leclère et al. 1994) and the slowdown of digestion and absorption of nutrients, associated with a modification in the viscosity of gastric and intestinal lumen (Schneeman 1982), and inhibition of the secretion of gut hormones (Morgan 1979) are thought to be important. Theses studies were mostly conducted using large amounts of fiber consumption, usually >20g/day. Therefore some authors consider it to be difficult to achieve such intakes of fiber from foods alone and fiber supplements are needed (Horton and Napoli 1996). There are also questions as to whether or not fiber plays a significant role (Nutall 1993).

On the other hand, newly manufactured dietary fiber from rice bran, BioBran/MGN-3, is used for general health promotion benefits, as a natural food supplement. This is also recognized to have an immunotherapeutic effect in the treatment of cancer patients (Ghoneum 1998; Ghoneum 1998).

Our previous study demonstrated that the administration of BioBran/MGN-3 improves streptozotocin (STZ)-induced diabetes (Ohara et al. 2000). Serum levels of triglycerides and total cholesterol were reduced by the administration of BioBran/MGN-3. Water intake was also reduced by the BioBran/MGN-3, which suggests that polyurea induced by STZ improved.

The present study was designed to examine the effect of BioBran/MGN-3 on glucose tolerance in the adult rat model of NIDDM as a result of neonatal administration of STZ.

Materials and Methods

1. Animals and Diets

Pregnant albino Wistar rats were obtained from Japan SLC (Hamamatsu, Japan). Animals bred in our colony were fed ad libitum with a commercial stock diet (MF; Oriental Yeast Co. Ltd., Osaka, Japan).

Male rats were made diabetic by a single intraperitoneal injection of 100 mg/kg STZ (Wako, Richmond, VA) (Blondel et al. 1989; Weir et al. 1981; Malaisse et al. 1991), dissolved in 20 mM citrate buffer pH 4.5 at 1.5 days of age. Non-diabetic control rats were injected with the buffer only. Animals were weaned on day 28, housed in individual stainless steel wire mesh cages (21×24×20 cm) in a well-ventilated room at 22±1℃ with the relative humidity at 40 to 60% and a 12-hour light/dark cycle. At eight weeks following weaning, diabetic rats were divided into 2 groups, those fed a 1.7% cellulose diet and those fed a 1% BioBran/MGN-3 diet (+0.7% cellulose) (**Table 1**). Non-diabetic control rats were also fed the 1.7% cellu-

Table 1 Composition of experimental diets

Ingredient	Cellulose diet (g/kg)	MGN-3
PEP*	80	0
α-Cornstarch	815	815
Mineral mixture**	35	35
Corn oil	50	50
Vitamin mixture***	10	10
Cellulose powder	10	—
MGN-3	—	10
Total dietary fiber	17	17

*PEP=Purified egg protein. (Taiyo Kagaku Co. Ltd., Mie, Japan)

**g/kg mixture; $CaHPO_4 \cdot H_2O$, 145.6; KH_2PO_4, 257.2; NaH_2PO_4, 93.5; NaCl, 46.6; Ca-lactate, 350.9; Fe-citrate, 318; $MgSO_4$, 71.7; $ZnCO_3$, 1.1; $MnSO_4 \cdot 5H_2O$, 0.3; KI, 0.1. (Oriental Yeast Co. Ltd., Osaka, Japan)

***g/kg mixture; all-rac-α-tocopheryl acetate, 5.0; cholecalciferol, 0.0025; menadione, 5.2; retinol acetate, 1.0; ascorbic acid, 30.0; calcium pantothenate, 5.0; choline chloride, 200.0, cyanocobalalmin, 0.0005; D-biotin, 0.02; folic acid, 0.2; inositol, 6.0; nicotinic acid, 6.0; pyridoxine hydrohloride, 0.8; p-aminobenzoic acid, 5.0; riboflavin, 4.0; thiamin hydrochloride, 1.2; Sufficient cellulose powder was added to make up 1 kg. (Oriental Yeast Co. Ltd., Osaka, Japan)

lose diet. Individual body weight and diet and water intakes were measured daily. The food and water intakes were measured by taking the weight of the day before from the next day. Animals were fed experimental diets and water ad libitum throughout the experimental period (60 days).

2. Dietary Fiber Supplement

BioBran/MGN-3 is a water soluble hemicellulose B that is enzymatically treated by carbohydrases complex, including $\alpha \cdot \beta$-glucosidase, $\alpha \cdot \beta$-galactosidase and β-1,4-glucosidase, from forest mushroom, *Lentinus edodes*. The main ingredient is an arabinoxylan; which specifies saccharides by anthronesulfuric acid method; 55-56%, protein by Lowry method; 10-20%, crude ash; 6-12% and moisture; less than 7%. MGN-3 is commercially known as BioBran (Daiwa Pharmaceutical Co. Ltd., Tokyo Japan).

3. Oral Glucose Tolerance Tests

Oral glucose tolerance tests were performed on day 58 after 20 hours of fasting. Blood was withdrawn from the tail vein at 0, 30, 60 and 120 minutes following the administration of 2g/kg glucose by stomach tube. Blood samples were immediately centrifuged at 4℃, and plasma was stored at -20℃ pending the analysis of glucose concentrations.

4. Plasma Biochemical Analysis

As anesthetics have an action of elevating blood glucose levels (unpublished observation), especially ether, collection of blood should be done by without anesthesia. Then the blood was collected by decapitation on day 60 after 20 hours of fasting. Blood was centrifuged at 600 ×g for 10 minutes. The separated plasma was protected from light and stored at -20℃ pending analysis.

Table 2 Body weight, food intake, feed efficiency and water intake in non-diabetic control rats fed the experimental 1% cellulose, diabetic rats fed 1.7% cellulose and diabetic fed 1% MGN-3 (+0.7% cellulose) diets for 60 days

Dietary group	n	Body weight (g)			Cumulative food intake (g)	Feed efficiency (%)	Cumulative water intake (g)
		Initial	Final	Body weight gain			
Non-diabetic control rats fed 1.7% cellulose	5	218±8[a]	337±12[a]	130±3	1143±30	10.9±0.3	1051±32[a]
Diabetic rats fed 1.7% cellulose	7	182±5[b]	297±10[b]	115±6	1085±19	10.6±0.4	924±34[b]
Diabetic rats fed 1% MGN-3(+0.7% cellulose)	8	180±9[b]	293±14[b]	113±14	1066±31	10.6±0.4	935±31[b]

Values are mean±SEM for 5-8 rats.
In each column, values not sharing a common superscript letter are significantly different at $p<0.05$.
Feed efficiency is body weight gain divided by food intake.

Plasma samples were analyzed for glucose (Trinder 1969), insulin (Mukoujima 1979), triglycerides (Spayd et al. 1978), total cholesterol (Allain et al. 1974), HDL-cholesterol (Ash and Hentschel 1978), albumin (Doumas et al. 1971), and urea nitrogen (Kanai and Kanai 1983) by commercial kits (Wako Pure Chemicals Co. Ltd., Osaka, Japan). Total protein concentration was measured using a color-imetric method, Coomassie protein assay (Pierce, Rockford, IL).

5. Data Analysis

Data are expressed as mean±SEM. All data were tested statistically using one-way analysis of variance (ANOVA) followed by Scheffé *F* test of multiple comparisons between pairs of means as appropriate at a significance level of 5%.

Results

1. Body Weight, Food Intake, Feed Efficiency and Water Consumption in Rats Fed Experimental Diets for 60 Days

Although the NIDDM rats showed the retardation of growth, body weight gain during the experimental period was not statistically different between groups (**Table 2**). Also, food intakes and feed efficiencies were not significantly different. Water consumption in the rats fed control diet was significantly more than that of diabetic rats, but these were within normal range.

2. Oral Glucose Tolerance Test

At any time following the administration of glucose, plasma glucose levels in the NIDDM rats were significantly higher than those in the non-diabetic controls (**Fig. 1**). In the NIDDM rats fed BioBran/MGN-3 diets, the significant and rapid rise of plasma glucose levels due to STZ was attenuated at 30 min.

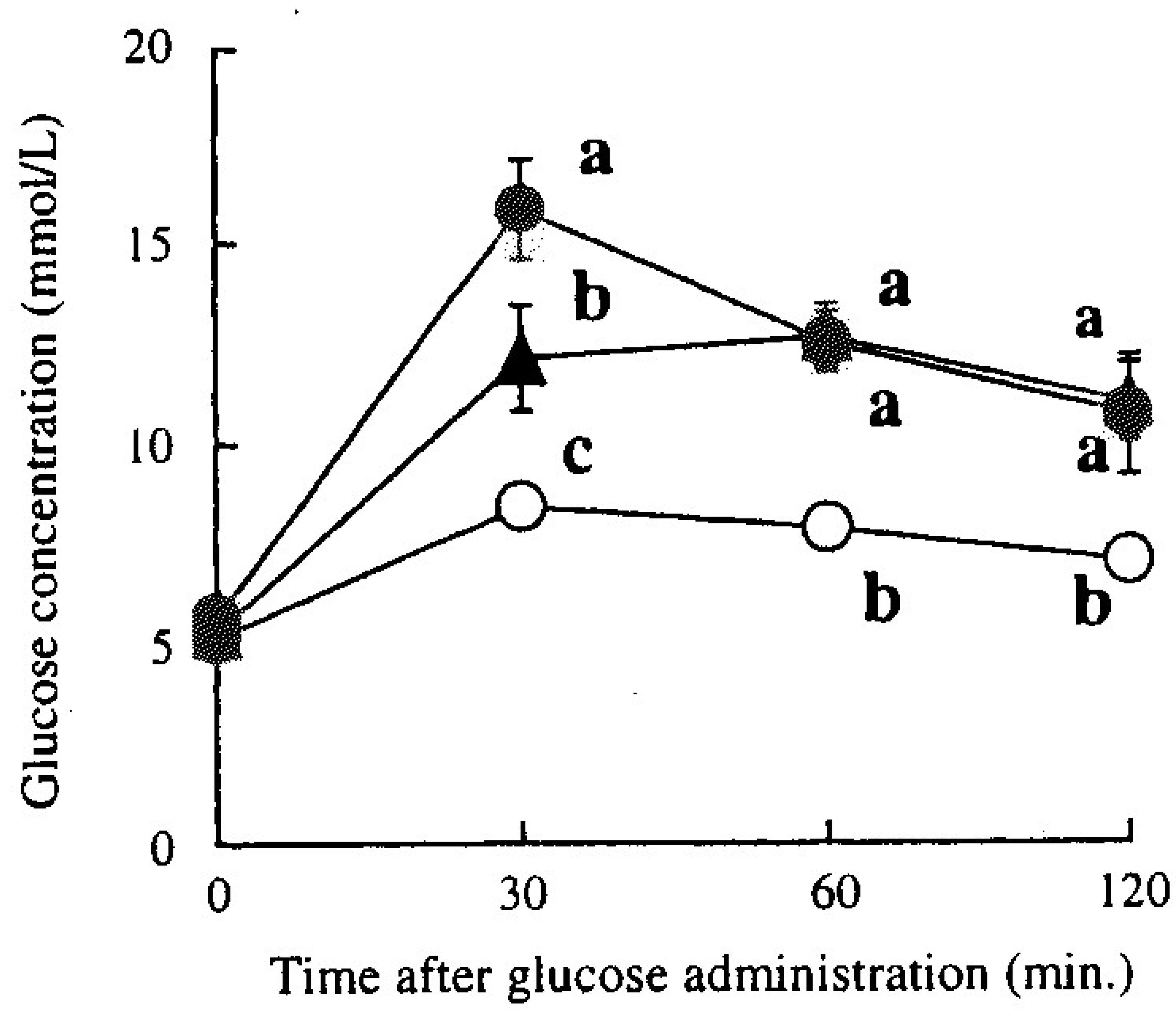

Fig.1 Glucose tolerance responses to glucose (2g/kg stomach tube) in non-diabetic male Wistar rats fed 1% cellulose diet (open circle), diabetic rats fed 1% cellulose diet (closed circle) and diabetic rats fed 1% MGN-3 (+0.7% cellulose) diet (triangle), measured on day 58 after 20 hours of starvation.

Each plotted point represents the mean for 5-8 rats and the vertical bars denote ±SEM.

At each time, values not sharing a common superscript letter are significantly different at $p<0.05$.

Table 3 Plasma biochemical values in fasting non-diabetic control rats fed the experimental 1.7% cellulose, diabetic rats fed 1.7% cellulose and diabetic rats fed 1% MGN-3 (+0.7% cellulose) diets for 60 days

	n	Glucose (mmol/L)	Insulin (μU/ml)	Triglycerides (mmol/L)	Total cholesterol (mmol/L)	HDL-cholesterol (mmol/L)	Urea nitrogen (mmol/L)	Total protein (g/dl)	Albumin (g/dl)
Non-diabetic control rats fed 1.7% cellulose	5	6.91±0.25	10.99±3.21	1.80±0.17	1.87±0.08^{b}	1.40±0.11	9.9±0.7^{b}	5.67±0.08	4.00±0.11
Diabetic rats fed 1.7% cellulose	7	7.81±0.4	9.60±2.2	1.89±0.23	2.24±0.14^{a}	1.70±0.14	13.3±1.3^{a}	5.71±0.1	4.09±0.03
Diabetic rats fed 1% MGN-3 (+0.7%cellulose)	8	7.10±0.7	15.82±1.8	2.10±0.15	1.97±0.11^{b}	1.56±0.10	13.4±0.9^{a}	5.61±0.1	4.14±0.06

Values are mean±SEM for 5-8 rats per group.

In each column, values not sharing a common superscript letter are significantly different at $P<0.05$.

3. Plasma Biochemical Analysis

Insulin concentration in the plasma of cellulose-fed diabetic rats were lower than those in non-diabetic controls, but these tended to be higher with administration of BioBran/MGN-3 although these values were not significantly different (**Table 3**). Plasma glucose levels in the fasting state were not different between groups. Among other biochemical measurements, total cholesterol and urea nitrogen were significantly higher in the NIDDM rats than those in the non-diabetic controls. The increase in total cholesterol levels was significantly attenuated by the administration of BioBran/MGN-3.

Discussion

β-Cells of the pancreas in the animals injected STZ were destroyed selectively, which led to insulin-deficient state (Steiner 1970; Hoftiezer and Carpenter 1973). As the β-cells have an ability to regenerate when the animals are 1 to 2 days after birth, severe insulin deficiency may not occur following injection of STZ to neonatal animals. In the case of rats, insulin content of the pancreas recovers to 50% of the control level within 2 weeks (Cantenys et al. 1981; Aratan-Spire et al. 1984). Plasma insulin concentration is normal after weaning and glucose concentration increases a little, but responses to glucose tolerance worsen significantly. The animals are known to resemble to human NIDDM. The results of the present study indicate that the rats had less severe NIDDM (**Table 2**). We infer that their pancreatic β-cells continued partial function. Although growth rates in the diabetic rats were significantly retarded, plasma glucose and insulin concentrations were close to normal. Water consumption was also normal, although normal rats drank significantly more water than the diabetic rats.

The results of this study indicate that BioBran/MGN-3 can modify the response to a glucose tolerance test in mildly NIDDM rats (**Fig. 1**). Many researchers have observed an improvement of postprandial glycemic response after incremental amounts of non-purified dietary fiber in NIDDM subjects (Guévin et al. 1996; Del Toma et al. 1988)

Generally, the main mechanisms cited to explain the effects of dietary fiber on the digestion and absorption of glucose are associated with a modification in the viscosity of gastric and intestinal lumen. The more that viscous dietary fiber exists in the gastric and intestinal lumen, the less effective is digestion and absorption. As a result, the reduction of postprandial blood glucose response would be expected.

Other aspects that could modify the viscosity of dietary fiber, such as soluble-in-soluble ratio and the total quantity of fiber (Guévin et al. 1996) have been reported.

BioBran/MGN-3 used in this study, is previously hydrolyzed by mushroom hydrases and the particle sizes become smaller than unpurified rice bran. Then, there is a possibility that portions of low molecular weight in this dietary fiber can be transported through the enterocyte brush border, and that these work directly on the pancreas and/or the receptor site of insulin action. Moreover, the improvement of BioBran /MGN-3 on diabetes observed by the addition of these amounts to approximately one-fifth or one-tenth the weight of other usual supplements. Therefore, differing mechanisms than the modification in the viscosity of gastric and intestinal lumen may account for improvements in glucose tolerance tests in diabetic rats.

Total cholesterol in the plasma of the diabetic rats in the present study clearly increased (**Table 3**). Plasma triglycerides and HDL-cholesterol concentrations in the diabetic rats also tended to increase, but was not significant. The addition of BioBran/MGN-3 attenuated the total-cholesterol increase in the plasma of the diabetic rats. Generally, a cholesterol lowering effect of dietary fiber is well known (Sugano et al. 1988; Demigné et al. 1998; Aoe et al. 1988), and has been believed to occur by inhibiting micellation of cholesterol, because soluble dietary fiber binds to bile acid and/or modifies enterohepatic circulation of bile acid. These studies are usually conducted with a diet containing over 5% dietary fiber (Sugano et al. 1988; Nagata et al. 1995). On the contrary, we added only 1% BioBran/MGN-3 to the diet. Therefore the mechanisms for reducing cholesterol levels in the plasma of the present study are thought to be quite different from other studies.

Rice bran is a byproduct of rice polishment and is usually removed from food because of its unpleasant taste. They are widely believed to be useless, although part of them is used for fertilizer and feeds for animals. However, rice bran contains many functional materials. The present study demonstrated that one of these functions is hypoglycemic activity.

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