

**Effects of Modified Arabinoxylan
from Rice Bran (BioBran/MGN-3)
on Serum Lipids and Taste Preference
in Streptozotocin-induced Diabetic Rats**

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Dietary Fiber, Serum Lipids, Taste preference, Diabetic Rat

Abstract

The present study was designed to determine whether or not the administration of BioBran/MGN-3 could improve streptozotocin (STZ) -induced diabetes. Taste preferences were also compared in diabetic and control rats. Male Sprague-Dawley rats were divided into control and diabetic groups. A single STZ injection, 65 mg per kg body mass i.p., induced diabetes. Rats were given free access to commercial diet and water for 2 months and BioBran/MGN-3 (0.5 g per kg body mass) was administered daily by stomach tube. Two-bottle-choice preference tests between aqueous solutions of either 5 mM citric acid, 27 mM monosodium glutamate, 0.016 mM quinine, or 0.82 mM saccharin in deionized water were conducted in the experimental period. Blood was collected and serum levels of glucose, insulin, triglycerides, total cholesterol, HDL-cholesterol, urea nitrogen, total protein, albumin, and zinc were measured. Serum triglycerides and

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total cholesterol decreased with the administration of BioBran/MGN-3, although serum insulin and glucose remained low and high, respectively. Water intake was also reduced by the BioBran/MGN-3, which suggests that polyuria induced by STZ improved. Diabetic rats showed significant aversion to citric acid and quinine when compared with control rats. BioBran/MGN-3 can be useful as a dietary fiber supplement for the treatment of diabetes. In addition, high taste sensitivity for sourness and/or bitterness is a characteristic of STZ-induced diabetes.

Introduction

Changes in concentrations of plasma lipids including cholesterol are complications frequently observed in patients with diabetes mellitus^{1,2)} and certainly contributes to the development of vascular disease in these patients. Many studies have been performed using diabetic animal models such as streptozotocin (STZ)-induced diabetic rats to clarify the mechanism by which the diabetic state induces hypercholesterolemia, or to confirm successful treatment of this disease³⁻⁶⁾.

Beneficial actions of diets high in fiber on the amelioration of diabetic symptoms are well documented. For example, certain dietary fibers, especially soluble ones, lower plasma cholesterol and maintain blood glucose concentrations within a suitable range⁷⁻⁹⁾. Mechanisms underlying these effects are not fully understood, but a delay of gastric emptying¹⁰⁾, interferences with intestinal absorption of cholesterol and glucose, and inhibition of digestive enzymes¹¹⁾ are thought to be caused. Most of these studies were conducted with large amounts of fiber consumption, usually >20g/day. Since, it is thought to be difficult to achieve such intakes of fiber from foods alone, fiber supplements are needed¹²⁾. There are also questions as to whether or not fiber plays a significant practical role¹³⁾.

On the other hand, disturbances of taste preference have been reported in diabetes, both in experimental animals¹⁴⁻¹⁶⁾ and in humans¹⁷⁻¹⁹⁾. Threshold values for sweetness are higher in diabetic patients than in normal subjects. However, there is a paucity of data available concerning other tastes, such as bitterness, saltiness, or sourness, with diabetes. In addition, few studies have been reported in the past on taste impairment and diabetes¹⁹⁾.

Newly manufactured dietary fiber, BioBran/MGN-3, effectively enhances natural killer cell activity and has an immunotherapeutic effect in the treatment of cancer patients^{20,21)}. However, the improvement of diabetic symptoms following BioBran/MGN-3 supplementation has not been investigated. Accordingly, the present study was designed firstly to examine the therapeutic effect of BioBran/MGN-3 on diabetes and secondly to measure taste preference of diabetic rats.

Materials and Methods

1. Animals

Eight-week-old male Sprague Dawley rats were obtained from Japan SLC (Hamamatsu, Japan). Animals were acclimated for 1 week and housed in individual stainless steel wire mesh cages (21×24×20 cm) in a well-ventilated room at 22±1°C with the relative humidity of 40 to 60% and a 12-hour light/dark cycle. Diabetes was induced by a single intraperitoneal injection of STZ (65 mg/kg) (Wako, Richmond, VA, USA), dissolved in 20mM citrate buffer pH 4.5. Non-Diabetic control rats were injected the buffer only. Rats were fed commercial stock diet (MF : Oriental Yeast Co., Ltd, Osaka, Japan) and water ad libitum

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throughout the experimental period (60 days). Individual body mass was measured daily. Blood samples were collected by decapitation on Day 60, centrifuged at 600×g for 10 minutes, separated sera were protected from light and stored at -20°C pending analysis. The experimental protocol was approved by the Animal Care and Use Committee of Kobe Women's University.

2. Dietary fiber supplement

BioBran/MGN-3 is an arabinoxylan extracted from rice bran that is treated enzymatically with an extract from *Basidiomycetes* mycelia. It is a hemicellulose that contains β -1,4 xylopyranose. MGN-3 is commercially known as BioBran (Daiwa Pharmaceutical Co., Ltd., Tokyo, Japan).

BioBran/MGN-3 mixed with 0.5% sodium alginate as a suspension stabilizer in order to prevent plugging of the syringe by the supplement, was administered (0.50 g/kg body mass) daily by stomach tube. Rats administered 0.5% sodium alginate alone were used as a vehicle control.

3. Preference tests

Two-bottle-choice preference tests were performed for 8 hours using an aqueous solution of either 5 mM citric acid (sour) on Days 49 and 59; 0.82 mM sodium saccharin (sweet) on Days 50 and 56; 0.016 mM quinine sulfate (bitter) on Days 51 and 55; or 27 mM monosodium glutamate (savory that is a taste quality represented typically by glutamates and 5'-nucleotides) on Days 53 and 58, and deionized water. The position of the flavored solutions in the cage was alternated after each measurement period. Taste preferences, expressed as percentages, were calculated according to the following formula:

$$\text{Preference (\%)} = \frac{\text{Vol. (ml) Test solution consumed}}{\text{Vol. (ml) Test solution} + \text{Vol. (ml) Water consumed}} \times 100$$

Presented data are means of two taste tests. The number of animals per group varied from 5 to 6 for each taste test. The same animals were used in all experiments.

4. Biochemical analysis

Serum samples were analyzed for glucose, insulin, triglycerides, total cholesterol, HDL-cholesterol, albumin, urea nitrogen, and zinc by commercial kits obtained from Wako Pure Chemicals (Code #271-31401, 305-11511, 276-69801, 272-64901, 274-67401, 274-24801, 279-36201 and 431-14901 respectively). Total serum protein concentration was measured using a colorimetric method, Coomassie protein assay reagent (Pierce, Rockford, Illinois, USA: Code #23200).

5. Data analysis

Data are expressed as mean $\pm$ SEM. These data were analyzed statistically by one-way analysis of variance²²⁾. A probability of 0.05 or less indicated significant difference.

Results

Following injection with STZ, these animals displayed the expected symptoms of insulin-dependent diabetes mellitus, i.e. hyperglycemia of more than 30 mmol/L within 48 hours, which persisted throughout the 60 day study period, depression of body mass gain (Table 1), and polydipsia (Table 2). Insulin con-

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Table 1 Body mass and serum biochemical values in MGN-3 fed non-diabetic and STZ-diabetic rats*

	Non-Diabetic		STZ-Diabetic	
	−MGN-3	+MGN-3	−MGN-3	+MGN-3
n	6	5	5	6
Body Mass (g)	496±6 ^a	471±7 ^a	228±11 ^b	276±29 ^b
Glucose (mmol/L)	8.31±0.27 ^b	7.70±0.21 ^b	34.00±2.09 ^a	33.84±2.13 ^a
Insulin (μU/ml)	40.1±4.8 ^a	37.6±6.3 ^a	0.0±0.0 ^b	2.1±1.3 ^b
Triglycerides (mmol/L)	2.32±0.19 ^c	1.98±0.25 ^c	23.82±6.15 ^a	11.74±3.26 ^b
Total Cholesterol (mmol/L)	2.08±0.09 ^b	1.94±0.14 ^b	5.71±1.52 ^a	3.11±0.73 ^b
HDL-Cholesterol (mmol/L)	1.45±0.09	1.25±0.08	1.78±0.30	1.74±0.35
Urea Nitrogen (mmol/L)	11.1±1.4 ^b	15.6±1.7 ^b	33.2±3.2 ^a	27.3±3.2 ^a
Total Protein (g/dl)	6.43±0.16 ^a	6.82±0.23 ^a	4.45±0.19 ^b	5.35±0.17 ^a
Albumin (g/dl)	3.84±0.03 ^a	3.74±0.08 ^a	2.54±0.06 ^b	2.64±0.10 ^b
Zinc (μmol/L)	9.15±0.35 ^c	9.79±0.65 ^{bc}	14.74±1.18 ^a	11.83±1.22 ^b

*Values are mean±SEM.

In each row, values not sharing a common superscript letter are significantly different at $p \leq 0.05$.

centrations in serum of diabetic rats were significantly lower than that in non-diabetic controls. Administration of BioBran/MGN-3, however, did not improve the hyperglycemia and hypoinsulinemia induced by STZ.

As shown in **Table 1**, serum triglycerides and total cholesterol levels were significantly higher in STZ-diabetic rats than in non-diabetic control animals. HDL-cholesterol levels, however, were unaffected by STZ injection or BioBran/MGN-3 administration. In diabetic animals, BioBran/MGN-3 reduced the rise in serum triglycerides and total cholesterol levels. In addition, serum urea nitrogen increased significantly and total protein and albumin levels decreased in STZ-diabetic rats compared to non-diabetic rats. When BioBran/MGN-3 was administered to diabetic rats, total protein levels recovered to near that of non-diabetic animals, although urea nitrogen was unaffected. Serum zinc concentration in STZ-diabetic rats were significantly higher than non-diabetic rats. Administration of BioBran/MGN-3 lowered serum zinc concentrations in STZ-diabetic rats. In non-diabetic animals, BioBran/MGN-3 administration had no effect on any of these parameters.

As shown in **Table 2**, total volume intake (intake of deionized water plus intake of flavored solution) for period of 8 hours increased significantly in the STZ-diabetic rats than in the non-diabetic rats. STZ-diabetic rats without BioBran/MGN-3 drank significantly more water than rats given BioBran/MGN-3. In the two-choice tests, all except non-diabetic control rats significantly preferred monosodium glutamate and all except non-diabetic given BioBran/MGN-3 rats significantly preferred saccharin. Whether BioBran/MGN-3 was administered or not, STZ-diabetic rats had a significantly decreased preference for citric acid and quinine solutions compared to non-diabetic rats.

Discussion

The present study was designed to investigate actions of BioBran/MGN-3 on serum biochemical param-

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Table 2 Total volume intake (intake of deionized water + intake of flavored solution) for 8 hours and percent preference (intake of flavored solution / total volume intake) in MGN-3 fed non-diabetic and STZ-diabetic rats*

	Non-Diabetic		STZ-Diabetic	
	-MGN-3	+MGN-3	-MGN-3	+MGN-3
5mM Citric Acid Total Intake (ml/100gBM)	0.9±0.2 ^c	1.1±0.1 ^c	28.4±4.3 ^a	15.0±2.3 ^b
Preference (%)	36.8±7.4 ^b	44.0±6.2 ^a	8.9±2.8 ^c	12.4±2.9 ^c
27mM Monosodium Total Intake (ml/100gBM)	Glutamate			
	1.2±0.2 ^c	1.3±0.1 ^c	32.7±4.8 ^a	19.3±2.9 ^b
Preference (%)	40.3±6.7 ^b	67.1±6.0 ^a	68.0±7.3 ^a	69.3±9.5 ^a
0.016mM Quinine Total Intake (ml/100gBM)	Sulfate			
	0.8±0.1 ^c	0.7±0.1 ^c	25.4±3.9 ^a	14.6±2.0 ^b
Preference (%)	25.2±6.8 ^b	35.4±9.7 ^a	17.9±9.0 ^c	10.1±3.4 ^d
0.82nM Sodium Total Intake (ml/100gBM)	Saccharin			
	1.0±0.2 ^c	1.4±0.2 ^c	27.6±4.6 ^a	14.0±1.8 ^b
Preference (%)	62.1±5.7 ^a	50.4±7.5 ^b	62.6±8.4 ^a	60.0±10.6 ^a

*Values are mean ±SEM for 5-6 rats per group.
In each row, values not sharing a common superscript letter are significantly different at $p \leq 0.05$.

eters and taste preference in STZ-induced diabetic rats and normal non-diabetic rats. A couple of days after a single injection of STZ rats showed clear signs of diabetic symptoms. Water intake increased markedly, rats failed to gain body mass, and blood glucose levels reached values of more than 30 mmol/L. These factors remained fairly constant for 60 days of subsequent testing.

Serum lipids in STZ-induced diabetic rats, especially, triglycerides and total cholesterol, were elevated significantly, together with significantly elevated serum glucose and decreased insulin concentrations. However, the administration of BioBran/MGN-3 markedly lowered triglycerides and total cholesterol concentrations. Although these results are consistent with previous reports²³⁾, there is considerable difference in the consumption of dietary fiber. The fiber level we used was only 0.5 g/kg/day and this amounts to approximately one-fifth to one-tenth of the usual supplement. Therefore, differing mechanisms other than the delay of gastric emptying¹⁰⁾ and/or interferences with intestinal absorption of cholesterol and inhibition of digestive enzymes¹¹⁾ may account for improvements in serum lipids profile in diabetic rats, although the mechanism by which BioBran/MGN-3 administration lowered serum triglycerides and total cholesterol was not specifically addressed in this study.

Serum glucose and insulin levels in STZ-induced diabetic rats remained as they were whether BioBran/MGN-3 was given or not. This means that the destruction of pancreatic β -cells by STZ in the current study was severe. The effect of destruction of fewer pancreatic β -cells remains to be examined.

Diabetes mellitus is characterized by derangement in metabolism not only of glucose and fat but also of protein²⁴⁾. However, protein has always received less attention than fat and glucose, both for alterations in its metabolism and in its nutritional implications. The present study examined the influence of diabetes on

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urea nitrogen, total protein, and albumin in serum. Results show that there were significant reductions in concentrations of total proteins and albumin and an elevation in the concentration of urea nitrogen. Significant improvement in concentration of total protein was observed by supplementation with BioBran/MGN-3, while concentrations of urea nitrogen and albumin remained unaffected. Gougeon et al.²⁴⁾ stated that protein intake as well as carbohydrate intake should be restricted in diabetes to normalize protein metabolism, which may normalize glycemia, and prevent typical symptoms of diabetic renal disease, albuminuria²⁵⁾. It is possible that treatment with BioBran/MGN-3, instead of protein restriction, can be used to improve protein metabolism.

A number of investigators have reported changes in consumption of various flavored substances in diabetes. Most of these studies were conducted with sweet compounds, and found a reduction in the preference for sweet tasting compounds by the diabetic^{16, 17, 19)}. Preference tests performed in this experiment indicate that the STZ-induced diabetic condition alters sensitivity to sour and bitter among various tastes. To the best of our knowledge, this is the only study in which preference for acid and bitter compounds have been elevated in diabetic rats. Although rats in the present experiment did not avoid sweet, mean preference scores were not different compared to the non-diabetic control. This is possibly due to elevated water intake, 27.6 ml/100 g BM/8 hours (**Table 2**).

Mechanisms underlying changes in consumption patterns for various tastes in diabetes are unknown, but several possibilities such as changes in salivary composition or flow rate need to be considered. It is also possible that the alteration of zinc concentration in blood could have some effect on taste acuity due to a decrease in taste receptors (**Table 1**), since the importance of zinc on taste acuity is well recognized in humans²⁶⁾ and animals^{27, 28)}.

These data clearly show that the administration of BioBran/MGN-3 reduced water intake and the rise in serum triglycerides, total cholesterol, and markedly elevated serum total protein in diabetic rats. The precise mechanism by which BioBran/MGN-3 improves lipid and protein metabolism in diabetic rats is unclear. It may be possible that BioBran/MGN-3 improves host response to resist diabetes, considering BioBran/MGN-3 is known to enhance natural killer cell activity^{20, 21)}.

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