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愛知学泉大学

研究論集　第37号　抜刷

2002年12月

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Key words：modified rice bran　修飾米糠　　glucose tolerance　耐糖能
diabetic rat　糖尿病ラット

ABSTRACT

The effect of modified rice bran 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% modified rice bran (+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 higher levels than those of the normal control in an oral glucose tolerance test. In the NIDDM rats fed the modified rice bran 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 modified rice bran.

Modified rice bran holds promise as a dietary fiber supplement for the treatment of diabetes.

インスリン非依存型糖尿病（NIDDM）ラットにおける修飾米糠の耐糖能への効果を見た。

NIDDMラットは生後1.5日目にストレプトゾトシン（STZ）を100mg／kg腹腔内に注射した。離乳後，1.7%セルロース食を与えた健常対照群，1.7%セルロース食を与えた糖尿病群，1％修飾米糠食（＋0.7%セルロース）を与えた糖尿病群の3群に分け，自由摂食で60日間飼育した。実験食投与後60日目に断頭屠殺し，血中のインスリン，グルコース，トリグリセライド，総コレステロール，HDL-コレステロール，総タンパク質，アルブミンと尿素窒素濃度を測定した。

OGTTにおける血中グルコース濃度は，1.7%セルロース食を与えた糖尿病群では，健常対照群よりも急速に，高く上昇した。一方，修飾米糠食を与えた糖尿病群では，急激な血糖の上昇は抑制された。さらに，修飾米糠食を与えた糖尿病群では，総コレステロール濃度が著しく減少した。修飾米糠を連続的に投与することで，OGTTにおける血糖値の急激かつ高値の上昇は抑制された。また，血中総コレステロール濃度が著しく減少したことから，修飾米糠は，糖尿病の症状を改善するための食物繊維補助食品としての効果が期待できるということが示唆された。

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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 > 20 g/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, modified rice bran, arabinoxylan compound, 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 modified rice bran improves streptozotocin (STZ)-induced diabetes (Ohara et al. 2000). Serum levels of triglycerides and total cholesterol were reduced by the administration of modified rice bran. Water intake was also reduced by the modified rice bran, which suggests that polyurea induced by STZ improved.

The present study was designed to examine the effect of modified rice bran on glucose tolerance in the adult rat model of NIDDM as a result of neonatal administration of STZ.

MATERIALS AND METHODS

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% modified rice bran diet (+ 0.7% cellulose) (Table 1). Non-diabetic control rats were also fed the 1.7% cellulose 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).

Dietary Fiber Supplement

Modified rice bran is a water soluble hemicellulose B that is enzymatically treated

Table 1 Composition of Experimental Diets

Ingredient	Cellulose diet	Modified rice bran diet
	g/kg	
PEP[1]	80	80
α-Cornstarch	815	815
Mineral mixture[2]	35	35
Corn oil	50	50
Vitamin mixture[3]	10	10
Cellulose powder	10	—
MGN-3	—	10
Total dietary fiber	17	17

[1]PEP=Purified egg protein. (Taiyo Kagaku Co. Ltd., Mie, Japan)

[2]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; Kl, 0.1. (Orientl Yeast Co. Ltd., Osaka, Japan)

[3]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)

by carbohydrases complex, including α・β-glucosidase, α・β-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%. Modified rice bran is commercially known as Biobran (Daiwa Pharmaceutical Co. Ltd., Tokyo, Japan).

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 2 g/kg glucose by stomach tube. Blood samples were immediately centrifuged at 4℃, and plasma was stored at −20℃ pending the analysis of glucose concentrations.

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 x g for 10 minutes. The separated plasma was protected from light and stored at −20℃ pending analysis.

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 concentra-tion was measured using a colorimetric method, Coomassie protein assay (Pierce, Rockford, IL).

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

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 difierent. Water consumption in the rats fed control diet was significantly more than that of diabetic rats, but these were within normal range.

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 modified rice bran diets, the significant and rapid rise of plasma glucose levels due to STZ was attenuated at 30 min.

Plasma Biochemical Analysis

Insulin concentrations 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 modified rice bran, 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 modified rice bran.

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

Table 2 Body Weight, Food Intake, Feed Effciency and Water Intake in Non-Diabetic Control Rats Fed the Experimental 1% Cellulose, Diabetic Rats Fed 1.7% Cellulose and Diabetic Fed 1% Modified Rice Bran (+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-diabehc 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% modified rice bran (+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.

are known to resemble to human NIDDM. The results of the present study indicate that the rats had less severe NIDDM(Table2). 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 modified rice bran 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-insoluble ratio and the total quantity of fiber (Guévin et al. 1996) have been reported.

Modified rice bran, used in this study, is previously hydrolyzed by mushroom hydrases and the particle sizes become smaller than

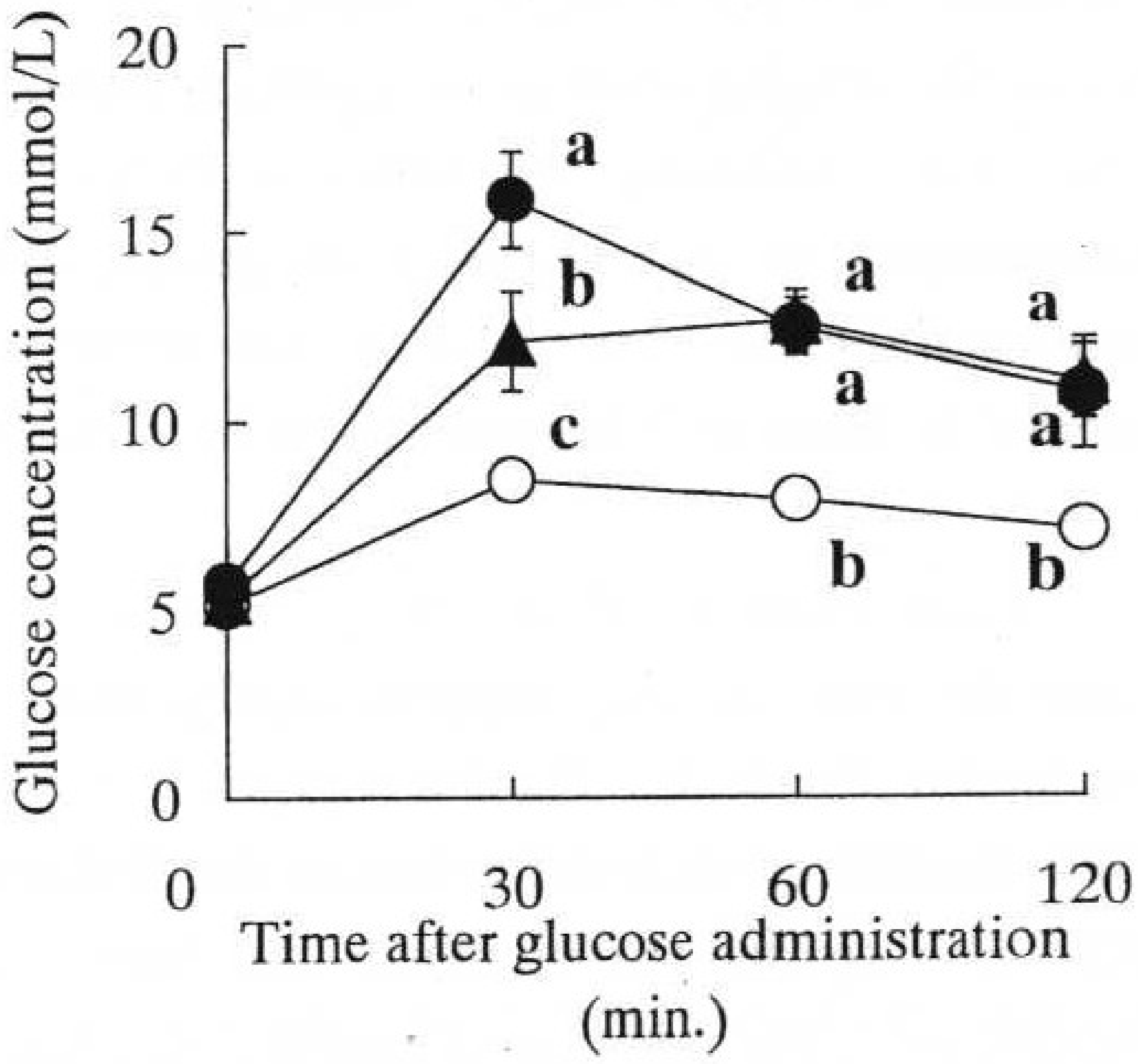

Fig. 1 Glucose tolerance responses to glucose (2g/kg stomach tube) in non-diabetic male Wistar rats fed 1% cellulose diet (open circle), diabetic rats fed 1% cellulose diet (closed circle) and diabetic rats fed 1% modified rice bran (+0.7% cellulose) diet (triangle), measured on day 58 after 20 hours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

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 modified rice bran on diabetes observed by the addition of these amounts to approximately one-fifih 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 modified rice bran 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% modified rice bran 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 fertlizer and feeds for animals. However, rice bran contains many functional materials. The present study demonstrated that one of these functions is hypoglycemic activity.

REFERENCES

Aoe, S., Ohta, T., and Ayano Y. (1988) Effect of rice bran hemicellulose on the cholesterol metabolism in rats (in Japanese), *J. Jpn. Soc. Nutr. Food Sci.*, 42, 55-61

Allain, C. C., Poon, L. S., Chan, C.S.G., Richmond, W. and Fu, P. C. (1974) Enzymatic determination of total serum cholesterol, *Clin. Chem.*, 20, 470-475

Anderson, J. W., Zeigler, J. A., Deakins, D. A., Floore, T. L., Dillon, D. W., Wood, C. L., Oeltgen, P. R. and Whitley, R. J. (1991) Metabolic effects of high-carbohydrate, high-fiber diets for insulin-dependent diabetic individuals, *Am. J. Clin. Nutr.*, 54, 936-943

Aratan-Spire, S., Wolf, B., Portha, B., Bailbe, D. and Czernichow, P. (1984) Streptozotocin treatment at birth induced a parallel depletion of thyrotropin-releasing hormone and insulin in the rat pancreas during development, *Endocrinol.*, 114, 2369-2373

Ash, K. O. and Hentschel, W. M. (1978) High-density lipoproteins estimated by an enzymatic cholesterol procedure, with a centrifugal analyzer. *Clin. Chem.*, 24, 2180-2184

Blondel, O., Bailbe, D. and Portha, B. (1989) Relation of insulin deficiency to impaired insulin action in NIDDM adult rats given streptozotocin as neonates, *Diabetes*, 38, 610-617

Cantenys, D., Portha, B., Dutrillaux, M. C., Hollande, E., Roze, C. and Picon, L. (1981) Histogenesis of the endocrine pancreas in newborn rats after destruction by streptozotocin. An immunocytochemical study, *Virchows Arch. B. Cell Pathol. Incl. Mol. Pathol.*, 35, 109-122

Del Toma, E., Clementi, A., Marcelli, M., Cappelloni, M. and Lintas, C. (1988) Food fiber choices for diabetic diets, *Am. J. Clin. Nutr.*, 47, 243-246

Demigné, C., Levrat, M. A., Behr, S. R., Moundras, C. and Rémésy, C. (1998) Cholesterol-lowering action of guar gum in the rat : changes in bile acids and sterols excretion and in enterohepatic cycling of bile acids, *Nutr. Res.*, 18, 1215-1225

Doumas, B. T., Watson, W. A. and Biggs, H. G.,(1971) Albumin standards and the measurement of serum albumin with bromcresol green. *Clin. Clim. Acta*, 31, 87-96

Ghoneum, M. (1998) Enhancement of human natural killer cell activity by modified arabinoxylane from rice bran (MGN-3), *Int. J. Immunotherapy,* 14, 89-99

Ghoneum, M. (1998) Anti-HIV activity in vitro of MGN-3, an activated arabinoxylane from rice bran, *Biochem. Biophys. Res. Comm.,* 243, 25-29

Goswamy, S. and Mani, U. V. (1985) Effect of wheat bran fibre on tissue lipids in diabetic rats, *Ind. J. Biochem. Biophys.,* 22, 240-243

Groop, P. H., Aro, A., Stenman, S. and Groop, L. (1993) Long-term effects of guar gum in subjects with non-insulin dependent diabetes mellitus, *Am. J. Clin. Nutr.,* 58, 513-518

Guévin, N., Jacques, H., Nadeau, A. and Galibois, I. (1996) Postprandial glucose, insulin, and lipid responses to four meals containing unpurified dietary fiber in non-insulin-dependent diabetes mellitus (NIDDM), hypertriglyceridemic subject, *J. Am. Coll. Nutr.,* 15, 389-396

Hoftiezer, V. and Carpenter, A. M. (1973) Comparison of streptozotocin and alloxan-induced diabetes in the rat, including volumetric guantitation of the pancreatic islets, *Diabetologia,* 9, 178-184

Horton, E. S. and Napoli, R. (1996) *Diabetes Mellitus, Present Knowledg of Nutrition,* 7th ed. Washington D. C., ILSI, 445-455

Kanai, I. and Kanai, M. (1983) *The manual of clinical examination* (in Japanese), 29. kinbara shuppann, 423-428

Leclère, C. J., Champ, M., Boillot, J., Guille, G., Lecannu, G., Molis, C., Bornet, F., Krempf, M., Delort-Laval, J. and Galmiiche, J. (1994) Role of viscous guar gums in lowering the glycemic response after a solid meal, *Am. J. Clin. Nutr.,* 59, 914-921

Malaisse, W. J., Giroix, M. -H., Zähner, D., Marynissen, G., Sener, A. and Portha, B. (1991) Neonatal streptozotocin injection, A model of glucotoxicity? *Metabolism,* 40, 1101--1105

Morgan, L. M., Goulder, T. J., Tsiolakis, D., Maeks, V., Alberti, K. G. M. M. (1979) The effect of unabsorbable carbohydrate on gut hormones, *Diabetologia,* 17, 85-89

Morgan, L. M., Tredger, J. A., Wright, J. and Marks, V. (1990) The effect of soluble-and insoluble-fibre supplementation on post-prandial glucose tolerance, insulin and gastric inhibitory polypeptide secretion in healthy subjects, *Br. J. Nutr.,* 64, 103-110

Mukoujima, T. (1979) Antibody and immune-complex (in Japanese), *Med. Tech.,* 7, 751-757

Nagata, J., Oku, H., Toda, T., Chinen, I. and Ohzeki, M. (1995) Physical properties and effects on lipid metabolism of soybean fiber in rats (in Japanese), *J. Jpn. Soc. Nutr. Food Sci.,* 48, 133-139

Nutall, F. Q. (1993) Dietary fiber in the management of diabetes, *Diabetes,* 42, 503-508

Ohara, I., Tabuchi, R. and Onai, K. (2000) Effects of modified rice bran on serum lipids and taste preference in streptozotocin-induced diabetic rats, *Nutr. Res.,* 20, 59-68

Schneeman, B. O. (1982) *Pancreatic and digestive function, Dietary Fiber in Health and Disease,* New York, Plenum Press, pp73-83

Spayd, R. W., Bruschi, B., Burdick, B. A., Dappen, G. M., Eikenberry, J. N., Esders, T. W., Figueras, J., Goodhue, C. T., LaRossa, D. D., Nelson, R. W., Rand, R. N., and Wu, T. W., (1978) Multilayer film elements for clinical analysis : Applications to representative chemical determinations, *Clin. Chem.,* 24, 1343-1350

Steiner, H., Olez, O., Zahnd, G. and Froesch, E. R. (1970) Studies on islet cell regeneration hyperplasia and intrainsular cellular interrelations in long lasting streptozotocin diabetes in rats, *Diabetologia,* 6, 558-564

Sugano, M., Nakano-Morita, M., Yoshida, K. and Imai, J. (1988) Influence of dietary apple and grape pomace on serum cholesterol level of the rat (in Japanese), *Nippon Shokuhin Kogyou Gakkaishi,* 35, 242-245

Trinder, P. (1969) Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen, *J. Clin, Path.,* 22, 158-161

Tsuji, K. (1988) Dietary fiber and health (in Japanese). *Jpn. J. Clin. Nutr.* 73, 673-680

Weir, G. C., Clore, E. T., Zmachinski, C. J. and Bonner-weir, S. (1981) Islet secretion in new experimental model for non-insulin-dependent-diabetes, *Diabetes,* 30, 590--595

Yuan, Z., He, P. and Takeuchi, H. (1998) Ameliorating effects of *Auricularia auricula-judae* Quel. on blood glucose level and insulin secretion in streptozotocin-induced diabetic rats (in Japanese), *J. Jpn. Soc. Nutr. Food Sci.,* 51, 129-133