Ameliorating Effects of Water-Soluble Polysaccharides from Woody Ear (*Auricularia auricula-judae* Quel.) in Genetically Diabetic KK-A^y Mice

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Summary We investigated the ameliorating effects of the three groups of water-soluble polysaccharides, a mixture of crude polysaccharides (FA), acidic polysaccharide fractions (FA-A), and neutral polysaccharide fractions (FA-N), obtained from the hot water extracts of the fruit bodies of *Auricularia auricula-judae* Quel. in genetically diabetic KK-A^y mice from 6 to 11 weeks of age. Male mice were divided into five dietary groups: 1) control group, given a basal diet; 2) FA group, given an FA diet (15 g FA/kg diet); 3) FA-A group, given an FA-A diet (8 g FA-A/kg diet); 4) low FA-N group, given a low FA-N diet (2 g FA-N/kg diet); and 5) high FA-N group, given a high FA-N diet (8 g FA-N/kg diet). Compared with the control diet, FA supplementation had significant effects in lowering fasting and nonfasting blood glucose, HbA_{1c}, urinary glucose, food intake, and water intake. FA administration also improved glucose tolerance to intraperitoneal glucose loading, but it did not affect the nonfasting insulin level. FA-N supplementation had dose-dependent effects in lowering fasting and nonfasting blood glucose, insulin, HbA_{1c}, urinary glucose, food intake, and water intake. However, the glucose tolerance was not ameliorated by either the low or the high FA-N diet. FA-A administration showed no beneficial effects in KK-A^y mice.

Key Words *Auricularia auricula-judae* Quel., water-soluble polysaccharides, KK-A^y mice, hypoglycemic effect

The fruit bodies of *Auricularia auricula-judae* Quel. (*A. auricula-judae*), family of *Heterobasidiaceae* (woody ears in English, mu-er in Chinese, and kikurage in Japanese), is among the most popular edible mushrooms in China and Japan, and since ancient times it also has been used to cure several diseases in China (1). We

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recently reported that dietary supplementation with the fruit bodies of *A. auricula-judae* at a dose of 50 g/kg of diet could lower fasting and postprandial blood glucose levels, increase insulin secretion, and ameliorate glucose tolerance to oral glucose loading in neonatally streptozotocin diabetic rat (2), an animal model of experimental noninsulin-dependent diabetes mellitus (NIDDM) with hypoinsulinemia and impaired glucose tolerance (3). In our previous studies, we found that dietary supplementation with the fruit bodies of *A. auricula-judae* at a dose of 50 g/kg of diet also could improve fasting blood glucose and reduce food intake in KK-A^y mice (unpublished data), an animal model of genetical NIDDM with hyperglycemia, hyperinsulinemia, glucosuria, and impaired glucose tolerance to intraperitoneal glucose loading (4). Furthermore, our previous studies also indicated that dietary supplementation with EtOH-soluble fractions, water-soluble low molecular weight fractions, and water-insoluble polysaccharide fractions from *A. auricula-judae* could not improve fasting blood glucose and reduce food intake in KK-A^y mice (unpublished data). In the present study, we try to learn whether water-soluble polysaccharides from *A. auricula-judae* would have beneficial effects on NIDDM.

According to the report of Sone et al, the water-soluble polysaccharides of *A. auricula-judae* contained a large amount of acidic heteropolysaccharide and a small amount of neutral β -D-glucan (5). The purpose of the present study is to examine the ameliorating effects of the three groups of water-soluble polysaccharides—a mixture of crude polysaccharides (FA), acidic polysaccharide fractions (FA-A), and neutral polysaccharide fractions (FA-N)—from the fruit bodies of *A. auricula-judae* in KK-A^y mice.

MATERIALS AND METHODS

Extraction and fractionation of the polysaccharides. The dried fruit bodies of *A. auricula-judae*, which were harvested in China and purchased at a market in Shizuoka City, Japan, were powdered by a mixer and passed through a 1 mm mesh sieve. As shown in Fig. 1, the powdered sample was extracted repeatedly with 85% ethanol at room temperature, and the resultant soluble substances were filtrated to remove components of low molecular weight. The residual material was extracted with boiling water and fractionated to give crude polysaccharide fractions (FA) in 25.5% yield. Then FA was further fractionated by treatment with cetylpyridinium chloride to give acidic polysaccharide fractions (FA-A) in 15.5% yield and neutral polysaccharide fractions (FA-N) in 2.6% yield. FA, FA-A, and FA-N were powdered with a mixer and used in this study.

Animal, diet, and experimental design. Thirty-five male KK-A^y/Ta Jcl mice, weighing 18–22 g, were purchased from Clea Japan (Tokyo, Japan) at 4 weeks of age. The experimental plan was approved by the Laboratory Animal Care Committee of the Faculty of Agriculture, Shizuoka University. The mice were individually housed in hanging stainless-steel wire cages and kept in an isolated

Fig. 1. Extraction and fractionation of water-soluble polysaccharides from fruit bodies of *Auricularia auricula-judae* Quel.

room with a controlled temperature (23–25°C) and controlled humidity (40–60%) and a 12 h cycle of light and dark (light on from 06:00 to 18:00). Table 1 shows the composition of experimental diets. The composition of basal diet (control diet) was based on the AIN-76™ diet (6). The FA, FA-A, and FA-N powders were added to the basal diet at the expense of cellulose. The amounts of FA, FA-A, and

Table 1. Composition of experimental diets.

Ingredient	Basal (control) diet	FA diet	FA-A diet (g/kg diet)	Low FA-N diet	High FA-N diet
Casein	200	200	200	200	200
DL-Methionine	3	3	3	3	3
α -Cornstarch	450	450	450	450	450
Sucrose	200	200	200	200	200
Corn oil	50	50	50	50	50
Mineral mixture ¹	35	35	35	35	35
Vitamin mixture ¹	10	10	10	10	10
Choline chloride	2	2	2	2	2
Cellulose	50	35	42	48	42
FA ²	0	15	0	0	0
FA-A ³	0	0	8	0	0
FA-N ⁴	0	0	0	2	8

¹ AIN-76™ (Nihon Nosan Kogyo, Yokohama, Japan).

² FA: the mixture of water-soluble crude polysaccharides from fruit bodies of *Auricularia auricula-judae*.

³ FA-A: water-soluble acidic polysaccharide fractions from fruit bodies of *Auricularia auricula-judae*.

⁴ FA-N: water-soluble neutral polysaccharide fractions from fruit bodies of *Auricularia auricula-judae*.

FA-N in their respective diets were to correspond to the amounts in the fruit bodies of *A. auricula-judae* at the dose of 50 g/kg of diet in our previous study. The amount of FA-N in the high FA-N diet was equivalent to the amount of FA-A in the FA-A diet.

The mice were fed a basal diet for 2 weeks. They were then randomly divided into 5 dietary groups of 7 mice each with similar mean body weights and 5 h fasting plasma glucose concentrations. The animals were allowed free access to the experimental diets and water for 5 weeks. Food consumption and water intake were recorded daily. Body weight and fasting plasma glucose were measured weekly in mice following 5 h of food deprivation. Blood samples were collected under nonanesthesia from the tail vein into heparinized capillary tubes (Funakoshi Pharmaceutical, Tokyo, Japan), and plasma was immediately separated by centrifugation ($9,000 \times g$, 5 min) to determine glucose concentration. Urinary glucose was investigated during the 5th week of feeding. Urine was collected over a 24 h period with 5 mL of 1 N hydrochloric acid to keep the urine from decaying, and the urinary sample was diluted to 500 mL for a determination of glucose concentration.

The intraperitoneal glucose tolerance test (IP-GTT) was performed in each mouse under nonanesthesia 5 weeks after feeding the experimental diets. The mice

Table 2. Body weight gain, food intake, and water intake in KK-A^y mice fed the experimental diets in Table 1 for 5 weeks.

Dietary group	Body weight gain	Food intake (g/35 d)	Water intake
Control	9.5 ± 0.2 ¹	193 ± 3 ^a	577 ± 36 ^a
FA	9.1 ± 0.3	180 ± 2 ^b	404 ± 28 ^b
FA-A	9.0 ± 0.2	199 ± 3 ^a	621 ± 22 ^a
Low FA-N	8.9 ± 0.5	182 ± 2 ^b	423 ± 32 ^b
High FA-N	8.3 ± 0.6	139 ± 4 ^c	206 ± 16 ^c

¹ Values are mean ± SE for 7 mice. Values in a column with different superscript letters are significantly different at $p < 0.05$.

received a 10% glucose solution (1 g glucose/kg body weight) intraperitoneally following 18 h of food deprivation. Blood samples were collected from the tail vein before and 30, 60, and 120 min after the loading of glucose. Plasma was immediately separated by centrifugation for measurement of glucose concentration.

On the final day of the experiment, the mice were sacrificed by decapitation under nonanesthesia and nonfasting. The blood samples were collected in polyethylene tubes with heparin for the measurement of HbA_{1c} levels and without heparin for the measurement of nonfasting serum glucose and insulin concentrations. Serum was harvested by centrifugation (2,000 × *g*, 20 min, 4°C).

Analytical procedures. The concentrations of blood glucose and urinary glucose were determined by the glucose oxidase method with a kit (Glucose C-II-Test, Wako Pure Chemical Industries, Osaka, Japan). Serum insulin level was determined by an enzyme-linked immunosorbent assay (ELISA) with a kit (Glazyme Insulin-EIA-Test, Wako Pure Chemical Industries). HbA_{1c} was measured by cation exchange column with a kit (Hemoglobin A_{1c} Micro Column Test, ROPET A_{1c}-S, Nippon Chemiphar, Tokyo, Japan).

Statistical analysis. Data in the experiment are showed as mean ± SE. All data were analyzed by one-way analysis of variance, and the differences between means were established by using Duncan's multiple-range test (7). The statistical significance of differences was established at $p < 0.05$.

RESULTS

Effect of polysaccharides on body weight, food intake, and water intake

The body weight gain, food intake, and water intake are presented in Table 2. The overall body weight gain tended to be reduced by supplementation of the water-soluble polysaccharides of *A. auricula-judae*, especially in mice fed the high FA-N diet, but no statistical differences were found among the 5 groups. When

Fig. 2. Effects of water-soluble polysaccharides from fruit bodies of *Auricularia auricula-judae* on plasma glucose concentrations following 5 h of food deprivation in KK-A^y mice. The mice were divided into five dietary groups and were fed the experimental diets in Table 1 for 5 weeks. The symbols and bars represent the mean and SE, respectively, for 7 mice. At each time, values with different letters are significantly different at $p < 0.05$.

overall means were compared, food intake and water intake were, respectively, about 7% and 30% lower in mice fed the FA diet, 6% and 27% lower in mice fed the low FA-N diet, and 28% and 64% lower in mice fed the high FA-N diet than in mice fed the control diet. No significant differences were observed in food intake and water intake between the control group and the FA-A group.

Effect of polysaccharides on fasting plasma glucose

Figure 2 shows the changes of 5 h fasting plasma glucose levels. Fasting plasma glucose concentrations in the control group and the FA-A group were gradually increased with age, and no significant differences were observed between the two groups. However, this rise was markedly suppressed by the FA diet and both FA-N diets compared with the control diet. In the FA group and the low FA-N group, the concentrations of fasting plasma glucose were kept at the initial levels throughout the whole period of experiment. In the high FA-N group, fasting plasma glucose levels were almost unchanged until the 3rd week of the experiment, and thereafter they were significantly lower than the initial value in the 4th and 5th weeks of the experiment ($p < 0.01$).

Effect of polysaccharides on glucose tolerance

Figure 3 shows the plasma glucose levels of the 5 dietary groups at various time intervals before and after IP-GTT. The blood glucose concentrations in the FA group were significantly lower before and 30, 60, and 120 min after the loading of glucose than in the control group. The delta area under the plasma glucose response curve (8) significantly decreased in the FA group (1.8 ± 0.2 mmol/L · h) compared

with that in the control group ($5.9 \pm 0.4 \text{ mmol/L} \cdot \text{h}$, $p < 0.01$). However, these differences were not significantly apparent in mice fed an FA-A or an FA-N diet (data not shown).

Fig. 3. Effects of water-soluble polysaccharides from fruit bodies of *Auricularia auricula-judae* on plasma glucose response to glucose tolerance test in KK-A^y mice. The mice were divided into five dietary groups. After being fed the experimental diets in Table 1 for 5 weeks, they were given 10% glucose solution (1 g/kg body weight) intraperitoneally following 18 h of food deprivation. The symbols and bars represent the mean and SE, respectively, for 7 mice. The value with an asterisk is significantly different from other values at the same time, $p < 0.05$.

Table 3. Urinary glucose, serum glucose, serum insulin, and HbA_{1c} levels in KK-A^y mice.¹

Dietary group	Urinary glucose (mmol/24 h/50 g)	Serum glucose (mmol/L)	Serum insulin (μU/mL)	HbA _{1c} (%)
Control	9.4 ± 0.6 ^{a,2}	46.0 ± 1.3 ^a	628 ± 59 ^a	3.4 ± 0.1 ^a
FA	4.0 ± 0.2 ^c	36.2 ± 2.4 ^b	660 ± 57 ^a	2.5 ± 0.3 ^{bc}
FA-A	9.7 ± 0.4 ^a	45.8 ± 1.4 ^a	625 ± 58 ^a	3.2 ± 0.3 ^{ab}
Low FA-N	6.8 ± 0.6 ^b	41.8 ± 1.3 ^a	584 ± 49 ^a	2.8 ± 0.2 ^{bc}
High FA-N	0.0 ± 0.0 ^d	13.9 ± 0.4 ^c	365 ± 28 ^b	1.9 ± 0.3 ^c

¹ The mice were divided into five dietary groups and fed the experimental diets in Table 1. Urine was collected over a 24 h period during the 5th week of feeding. After being fed the experimental diets for 5 weeks, the mice were sacrificed by decapitation under nonfasting. The blood samples were collected for the measurement of serum glucose, serum insulin, and HbA_{1c}.

² Values are mean ± SE for 7 mice. Values in a column with different superscript letters are significantly different at $p < 0.05$.

Effect of polysaccharides on urinary glucose, nonfasting serum glucose, nonfasting serum insulin, and HbA_{1c}

The concentrations of urinary glucose, nonfasting serum glucose, nonfasting serum insulin, and HbA_{1c} of 5 dietary groups are presented in Table 3. No significant differences were observed in these characteristics between the control and the FA-A groups.

Urinary glucose excretions were 58% lower in mice fed the FA diet and 28% lower in mice fed the low FA-N diet than in mice fed the control diet. Urinary glucose excretion was not detected in mice fed the high FA-N diet.

Nonfasting serum glucose and insulin concentrations were dramatically lower in mice fed the high FA-N diet than in mice fed the control diet, but these differences were not significantly apparent in mice fed the low FA-N diet. Nonfasting serum glucose levels were significantly lowered in mice fed the FA diet, but nonfasting serum insulin concentration was not changed compared with mice fed the control diet.

HbA_{1c} levels were significantly lowered by the FA diet and by both FA-N diets dose dependently compared with the control diet.

DISCUSSION

In this study, we examined the ameliorating effects of water-soluble polysaccharides FA, FA-A, and FA-N obtained from the hot-water extracts of the fruit bodies of *A. auricula-judae*, in genetically diabetic KK-A^y mice. This study showed that FA, FA-A, and FA-N had significantly different effects on glycemic control and food intake in KK-A^y mice.

FA is a crude polysaccharide fraction from fruit bodies of *A. auricula-judae* and is considered to be a mixture consisting mainly of acidic and neutral polysaccharides. The results of this study demonstrate that dietary supplementation with FA at a dose of 15 g/kg of diet could suppress the elevation of fasting and nonfasting blood glucose levels, reduce HbA_{1c} and urinary glucose concentrations, and improve glucose tolerance to IP-GTT in KK-A^y mice compared with the control diet. The results of this study also indicate that FA supplementation at a dose of 15 g/kg of diet caused a slight but significant reduction in food consumption, accompanied with a decreased water intake. These results suggest that water-soluble crude polysaccharides had significant effects on glycemic control and food intake in KK-A^y mice.

FA-N is a water-soluble neutral polysaccharide fraction from fruit bodies of *A. auricula-judae*. The results of this study showed that FA-N administration had dose-dependent effects in lowering fasting and nonfasting blood glucose, insulin, HbA_{1c}, urinary glucose, food intake, and water intake compared with the control diet, suggesting that water-soluble neutral polysaccharide may be a major active agent of *A. auricula-judae* for improving hyperglycemia, hyperinsulinemia, glucosuria, and hyperphagia of KK-A^y mice. It may be worthwhile noting that

FA-N intake, either a low or a high dose, did not ameliorate impaired glucose tolerance to IP-GTT in KK-A^y mice like FA did. At present, we have no definitive experimental evidence to explain this result. Hikino and Mizuno found that hypoglycemic activity was decreased during the purification of some polysaccharides from *Ganoderma lucidum*, a medicinal mushroom (9). Ukai also reported that an antiphlogistic effect of the crude polysaccharide from *A. auricula-judae* disappeared during the further fractionation of polysaccharides (10). Thus one possibility is that the ameliorating effect of *A. auricula-judae* on glucose tolerance to IP-GTT in KK-A^y mice was not contributed by water-soluble neutral polysaccharide alone.

Kiho et al (11) found that the water-soluble acidic polysaccharide from the fruit bodies of *Tremella fuciformis* (Yin-er in Chinese, shirokikurage in Japanese), along with *A. auricula-judae* belonging to the family *Heterobasidiaceae*, exhibited a significantly hypoglycemic action by a dose of 750 mg/kg/d in streptozotocin-induced diabetic mice, an animal model of insulin-dependent diabetes mellitus (IDDM) (12). The constitution of the water-soluble acidic polysaccharide from *Tremella fuciformis* has been reported to resemble that of the water-soluble acidic polysaccharide from *A. auricula-judae* (13). However, in contrast to the case of *Tremella fuciformis*, our present study showed that the administration of the acidic polysaccharide fractions from *A. auricula-judae* at a dose of 1,232 mg/kg/d showed no beneficial effects in KK-A^y mice, though FA-A was a major constituent of the water-soluble polysaccharides of *A. auricula-judae* and the dose of FA-A in the FA diet was the same as that of FA-N in the high FA-N diet.

The hypoglycemic action of dietary fiber to diabetes was often considered to be due to an increased consumption of dietary fiber in large quantities (14, 15). The American Diabetes Association has revised its position on medical nutrition therapy for people with diabetes mellitus. The 1994 recommendation for dietary fiber is the same for persons with or without diabetes. Daily consumption of a diet containing 20–35 g dietary fiber from a wide variety of food sources is recommended (14). Several previous studies using dietary fiber levels of 45–50 g/d showed little or no significant benefits to carbohydrate metabolism in patients with diabetes (16–18), and the greatest effects on carbohydrate metabolism have relied on dietary fiber levels from 70 to 100 g/d (19–21), but these levels of dietary fiber intake may be impossible to achieve consistently in a large segment of the population (15). On the other hand, most of the previous studies on dietary fiber in diabetic patients or diabetic animals used single-type dietary fiber such as water-soluble dietary fiber or water-insoluble dietary fiber as a food admixture. In our present study, the total amount of dietary fiber in FA and both FA-N diets was kept at the same level as that in the basal diet. When cellulose of the basal diet, a water-insoluble polysaccharide that has been indicated to have no hypoglycemic effect in KK-A^y mice (22), was partially exchanged by water-soluble polysaccharides FA or FA-N, significant beneficial effects were observed in KK-A^y mice. The effective dose in low FA-N diet is about 300 mg/kg/d. The results of the present study suggest that the hypoglycemic effect of dietary fiber might be related to its type rather than to the

amount consumed. Increasing the rate of some water-soluble dietary fibers, which have been confirmed to be effective in improving diabetic symptoms, in total dietary fiber intake might be more beneficial to diabetic patients for their dietary management than for only increasing dietary fiber consumption in large quantities.

Several studies reported that the reduction of food intake usually produced lower body weight in mouse and rat (23–25). In our present study, however, the reduction of food intake by the supplementation of FA or FA-N did not significantly affect the body weight gain of KK-A^y mice. This result may be due to the improvement of glucose utilization and/or metabolism in diabetic mice fed the FA or FA-N diet. On the other hand, it is likely that decreased calorie intake contributed to the ameliorating effects of water-soluble polysaccharides of *A. auricula-judae* on glycemic control in KK-A^y mice. Further experiments using a dietary restriction are needed to clarify this hypothesis.

KK-A^y mice had genetically determined hyperinsulinemia. In this study, FA-N was considered to be a major active agent of *A. auricula-judae* for improving hyperinsulinemia and showed a significantly dose-dependent trend. When mice were fed the high FA-N diet in which the dose of FA-N was about 4 times greater than in the fruit bodies of *A. auricula-judae* at a dose of 50 g/kg of diet, the nonfasting insulin level was dramatically lowered. In this study, FA administration improved nonfasting serum glucose concentration, but it did not affect the nonfasting serum insulin level. One reason for this insufficiency might be due to the FA dose used in this study, which was enough to improve hyperglycemia, but not enough to improve hyperinsulinemia of KK-A^y mice because the FA-N dose in the FA diet was insufficient. The administration of a low FA-N diet showed a similar result in the nonfasting serum insulin level, as the FA diet did.

At present, the mechanism of the ameliorating effects of water-soluble crude polysaccharide fractions and neutral polysaccharide fractions from *A. auricula-judae* in KK-A^y mice are unknown. Further studies are in progress.

In conclusion, this study demonstrates the ameliorating effects of the water-soluble polysaccharides from *A. auricula-judae* in KK-A^y mice, and it confirms that these beneficial effects on glycemic control and food intake are mainly contributed by the water-soluble neutral polysaccharides, but not by the water-soluble acidic polysaccharides. Water-soluble polysaccharides of *A. auricula-judae*, especially the water-soluble neutral polysaccharides, may be the good dietary fiber source in the dietary management of NIDDM and may have beneficial effects on improving diabetic symptoms.

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