Hypoglycemic Effect of Water-soluble Polysaccharide from *Auricularia auricula-judae* Quel. on Genetically Diabetic KK-A<sup>y</sup> Mice

Zuomin YUAN,*# Puming HE,** Jianhui CUI,** and Hisanao TAKEUCHI**

*The United Graduate School of Agricultural Science, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
**Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka 422-8529, Japan

Received April 8, 1998

The hypoglycemic effect of water-soluble polysaccharide (FA) from fruiting bodies of *Auricularia auricula-judae* Quel. was investigated on genetically diabetic mice (KK-A<sup>y</sup>) from 10 to 14 weeks of age. Male mice were divided into 3 groups, the control group and FA-fed group having free access to the control diet or FA diet (30 g of FA/kg of diet). The food-restricted group had restricted access to the control diet at the level of the diet consumed by the FA-fed group. Compared with the control group, FA supplementation had a significant effect in lowering plasma glucose, insulin, urinary glucose, and food intake. FA administration also increased the tolerance to intraperitoneal glucose loading and the hepatic glycogen content. In the food-restricted group, the reduced food intake slightly lowered the plasma and urinary glucose levels, but did not improve hyperinsulinemia and glucose tolerance. This study shows that FA had a hypoglycemic effect on KK-A<sup>y</sup> mice, and the reduced food consumption was not a major factor which contributed to the hypoglycemic action of FA.

**Key words:** hypoglycemic effect; water-soluble polysaccharide; *Auricularia auricula-judae* Quel.; KK-A<sup>y</sup> mice; restricted feeding

The addition of a certain type of dietary fiber to food, particularly some water-soluble dietary fibers such as guar gum and pectin, has been reported to show a hypoglycemic effect on diabetic patients and animals. However, the dietary fiber used in previous diabetic studies has chiefly come from higher plants. It is well known that edible mushrooms, which are universal fungi, have a high dietary fiber content. Although some polysaccharides from edible mushrooms have been reported to show a hypoglycemic effect on the diabetic rat or mouse, few edible mushrooms have been investigated in this respect.

Fruiting bodies of *Auricularia auricula-judae* Quel. (*A. auricula-judae*) of the Heterobasidiae family is an edible mushroom (woody ears in English, mu er in Chinese, and kikurage in Japanese). It has been widely used in China as a traditional food and also used to cure some diseases since ancient times. However, the hypoglycemic effect of water-soluble polysaccharides from the fruiting bodies of *A. auricula-judae* on diabetes remains unclear.

Many previous studies on the hypoglycemic effect of dietary fiber used streptozotocin-induced animals which is the model for insulin-dependent diabetes mellitus (IDDM). However, very few studies about the hypoglycemic effect of dietary fiber have used the animal model for non-insulin-dependent diabetes mellitus (NIDDM). Since NIDDM accounts for the great majority of individuals with diabetes, we think that it would be more interesting to know whether water-soluble polysaccharide from the fruiting bodies of *A. auricula-judae* would have a beneficial effect on NIDDM. In this study, KK-A<sup>y</sup> mice, one of the animal models for NIDDM, were used. KK-A<sup>y</sup> mice have genetically determined obesity and such diabetic syndromes as hyperglycemia, hyperinsulinemia, glucosuria, and severe insulin resistance, all of which increase with age until at least 16 weeks old. KK-A<sup>y</sup> mice also show impaired glucose tolerance to an intraperitoneal glucose administration.

In addition, previous studies in our laboratory have found that the food consumption and water intake by KK-A<sup>y</sup> mice were significantly higher than those of normal animals such as C57BL/J mice (He, P., et al., unpublished results).

The primary objective of this study is to investigate the hypoglycemic effect of water-soluble polysaccharide (FA) from fruiting bodies of *A. auricula-judae* on KK-A<sup>y</sup> mice from 10 to 14 weeks of age. The inhibitory effect of FA on food consumption is also examined to confirm whether reduced food intake would be a major factor to cause a hypoglycemic effect on diabetic animals.

**Materials and Methods**

Isolation of the polysaccharide. The water-soluble polysaccharide of *A. auricula-judae* was prepared according to the method reported by Mizuno et al.

Dried fruiting bodies of *A. auricula-judae*, which were harvested in China and obtained from a market in Japan, were powdered with a mixer, and the powder was passed through a 1-mm-mesh sieve. The powdered sample (400 g) was extracted with 4 l of 85% ethanol at room temperature for 48 h, and the soluble substances

---

* Present address: Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka 422-8529, Japan

Abbreviations: FA, water-soluble polysaccharide from *Auricularia auricula-judae* Quel.; *A. auricula-judae*, *Auricularia auricula-judae* Quel.; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus
were filtered to remove the components of low molecular weight. This process was conducted 3 times, and the resulting residue was extracted 4 times with hot water (12 l, heated in a boiling-water bath for 3 h each time). The extract was filtered through filter paper and concentrated under reduced pressure, before the polysaccharide in the extract was precipitated by adding five volumes of ethanol. The resulting precipitate was collected by centrifugation, washed with ethanol, dialyzed, and lyophilized to give 102 g of a crude polysaccharide fraction (FA) which was powdered with a mixer and for subsequent use in this study.

**Animals and diets.** Twenty-four male KK-A’/Ta JI mice were obtained from Clea Japan (Tokyo, Japan) at 6 weeks of age. The experimental plan was approved by the Laboratory Animal Care Committee of the Faculty of Agriculture at Shizuka University. The mice were individually housed in suspended stainless-steel wire cages and kept in an isolation room with a controlled temperature (23–25°C) and humidity (40–60%) and a 12 h cycle of light and dark (lights on from 06:00 to 18:00 h).

The composition of the basal diet (control diet) was based on the AIN-76™ formula and is indicated in Table 1. The FA powder was added to the basal diet at the expense of cellulose (FA diet, Table 1).

The mice were allowed free access to the basal diet and water for 4 weeks until reaching a high plasma glucose level. At 10 weeks of age, the mice were randomly divided into 3 groups of 8 mice each with similar mean weights and similar plasma glucose concentration. The control group had free access to the control diet, the FA-fed group had free access to the FA diet, and the food-restricted group had restricted access to the control diet at the level of the diet consumed by the FA-fed group. All animals had free access to water throughout the experiment.

**Animal experiments.** Food consumption and water intake were recorded daily. Body weight and fasting plasma glucose were measured weekly in the mice following 5 h of food deprivation. Blood samples were collected without anesthesia from the tail vein into heparinized capillary tubes (Funakoshi Pharmaceutical Co., Tokyo, Japan). Plasma was immediately separated by centrifugation (9000 × g for 5 min) to determine the fasting plasma glucose concentration. Urinary glucose was investigated before the experiment (at 10 weeks of age) and in the 4th week of feeding. Urine was collected over a 24 h period with 5 ml of 1 N hydrochloric acid to keep the urine from deteriorating. The urine sample was diluted to 500 ml for the determination of urinary glucose concentration.

After 4 weeks of feeding with the experimental diets, an intraperitoneal glucose tolerance test was performed without anesthesia. Each mouse received a 10% glucose solution (1 g of glucose/kg of body weight) intraperitoneally following 20 h of food deprivation. Blood samples were collected from the tail vein before administering the glucose and at 30, 60 and 120 min thereafter. Plasma was immediately separated by centrifugation for measurement of the plasma glucose concentration.

On the final day of the experiment, the mice of the control and food-restricted groups were fed on the corresponding control diet, and the mice of the FA-fed group were fed on the corresponding FA diet within 30 min following 20 h of food deprivation. Both corresponding diets contained 3 g of available carbohydrate/kg of body weight, respectively. The mice were then sacrificed by decapitation without anesthesia 120 min after the diets had been administered. Blood samples were collected in polyethylene tubes without heparin. Serum was harvested by centrifugation (2000 × g for 20 min at 4°C) to determine the postprandial serum glucose and insulin concentrations. The heart, lungs, liver, spleen, and kidneys were quickly removed and weighed, the organ weights being expressed as both absolute and relative weights. The liver was digested in a 30% potassium hydroxide solution, and the glycogen fraction was obtained by adding ethanol.

**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 Chemicals, Osaka, Japan). Serum insulin was measured with a kit (Glucylne Insulin-EIA-Test, Wako Pure Chemicals, Osaka, Japan). The amount of liver glycogen was analyzed by the method of Hassan and Abraham.

**Statistical analysis.** Data in the experiment are shown as the mean ± SEM. All data were analyzed by a one-way analysis of variance, and the differences between means were established by Duncan’s multiple-range test. The statistical significance of differences was established as p < 0.05.

**Results**

**Body weight, food intake, and water intake**

The body weight changes are presented in Fig. 1. There were no significant differences among the 3 groups. As shown in Fig. 2, the food intake and water intake by the FA-fed group were lower each week than

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Basal diet (control diet)</th>
<th>FA diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200 (g/kg of diet)</td>
<td>200</td>
</tr>
<tr>
<td>dL-Methionine</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>o-Corn starch</td>
<td>450</td>
<td>450</td>
</tr>
<tr>
<td>Sucrose</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mixture*</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mixture*</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>FA**</td>
<td>0</td>
<td>30</td>
</tr>
</tbody>
</table>

* AIN-76™ (Nihon Nosan Kogyo Ltd., Yokohama, Japan).
** FA: water-soluble polysaccharide from the fruiting bodies of Auricularia auricula-judae Quel.
those by the control group. When overall means (Table 2) were compared, the food intake and water intake were respectively about 14% and 29% lower in the mice fed on the FA diet than in the mice fed the control diet. Pair-feeding resulted in the food-restricted group having almost the same food intake and water intake as the FA-fed group throughout the experiment.

Fasting plasma glucose concentration
Changes in the 5 h fasting plasma glucose concentration are shown in Fig. 3. The fasting plasma glucose concentration in the control group gradually increased with age, but that in the FA-fed group dramatically decreased in the 1st and 2nd weeks of feeding, the values thereafter being almost constant until the 4th week of feeding. In the food-restricted group, the fasting plasma glucose level was slightly reduced during the first 3 weeks of feeding compared with the control group.

Table 2. Food Intake, Water Intake, Postprandial Serum Glucose Concentration, Postprandial Serum Insulin Concentration, Liver Glycogen Concentration, and Kidney Weights in KK-A' Mice Fed on the Experimental Diets in Table 1

<table>
<thead>
<tr>
<th>Dietary group*</th>
<th>Control</th>
<th>FA-fed</th>
<th>Food-restricted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/28 d)</td>
<td>171 ± 7**</td>
<td>147 ± 3b</td>
<td>146 ± 1b</td>
</tr>
<tr>
<td>Water intake (g/28 d)</td>
<td>748 ± 41a</td>
<td>529 ± 29b</td>
<td>530 ± 2b</td>
</tr>
<tr>
<td>Postprandial characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum glucose (mg/100 ml)</td>
<td>216.1 ± 13.2a</td>
<td>153.3 ± 9.6a</td>
<td>193.0 ± 11.2a</td>
</tr>
<tr>
<td>Serum insulin (μU/ml)</td>
<td>50.7 ± 3.6a</td>
<td>27.3 ± 2.2a</td>
<td>47.1 ± 3.0a</td>
</tr>
<tr>
<td>Liver glycogen (mg/g of wet liver)</td>
<td>23.6 ± 1.1b</td>
<td>31.7 ± 0.7a</td>
<td>27.5 ± 1.7b</td>
</tr>
<tr>
<td>Kidney weight (mg/50 g of body wt.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left kidney</td>
<td>367 ± 7a</td>
<td>313 ± 14b</td>
<td>341 ± 9b</td>
</tr>
<tr>
<td>Right kidney</td>
<td>371 ± 5a</td>
<td>321 ± 8b</td>
<td>363 ± 7b</td>
</tr>
</tbody>
</table>

* Refer to Fig. 1.
** Each value is the mean ± SEM for 8 mice. Values in a row with different superscript letters are significantly different at p < 0.05.
The effects of water-soluble polysaccharide from *Auricularia auricula-judae* and feeding restriction on the fasting plasma glucose concentration following 5 h of food deprivation in KK-A' mice were studied. The mice were fed on the non-restricted control diet (control group), the restricted control diet (food-restricted group), or the non-restricted FA diet (FA-fed group) for 4 weeks. The symbols and bars represent the mean and SEM, respectively, for 8 mice. At each time, values with different letters are significantly different at $p<0.05$.

### Urinary glucose excretion

The urinary glucose concentration changes are presented in Table 3. The urinary glucose concentrations of the 3 groups were similar before the experiment, but were significantly different by the 4th week of feeding. Compared with the initial value, the urinary glucose excretion in the 4th week of feeding had increased in the control group, decreased in the FA-fed group, and was constant in the food-restricted group.

### Postprandial serum glucose and insulin levels, hepatic glycogen content, and organ weights

As shown in Table 2, compared with the control group, the FA-fed group had significantly lower postprandial serum glucose and insulin levels; however, there were no such significant differences in postprandial serum glucose and insulin responses in the food-restricted group.

Table 2 shows that there was about a 31% increase in the hepatic glycogen content of the FA-fed group as compared to that of the control group, but this difference was not significant in the food-restricted group.

No significant differences were apparent in the absolute and relative weights of the heart, lungs, liver, and spleen (data not shown). However, both the absolute (data not shown) and relative weights of the left and right kidneys were higher in the control and food-restricted groups than in the FA-fed group (Table 2).

### Discussion

We investigated the hypoglycemic effect of water-soluble polysaccharide (FA) from fruiting bodies of *A. auricula-judae* in genetically diabetic KK-A' mice. The inhibitory effect of this extract on food consumption was also examined to determine whether reduced food intake would be a major factor to cause the hypoglycemic action in diabetic animals. The results of this study clearly...
demonstrate that dietary supplementation with FA at the 3% level could reduce the fasting plasma glucose concentration and urinary glucose excretion, suppress the postprandial high serum glucose and insulin levels, and improve the glucose tolerance in KK-A' mice. The results of this study also indicate that FA supplementation at the 3% level to the diet caused a slight decrease in the food consumption of KK-A' mice. This decreased food consumption was confirmed to be a major factor in the decreased water intake. This study also confirms that the reduced food consumption could slightly reduced the fasting plasma glucose and urinary glucose levels, but could not ameliorate the impaired glucose tolerance and hyperinsulinemia of KK-A' mice.

The hypoglycemic effect of FA on KK-A' mice may be partially related to its viscosity. A high-viscosity water-soluble dietary fiber such as guar gum or pectin, when added to food, has been reported to increase the gastric emptying time, and to suppress and/or delay the intestinal digestion and absorption of carbohydrate.\(^{14-16}\) On the other hand, Higgins et al. have reported that long-term consumption of a diet in which available carbohydrate was rapidly absorbed caused insulin resistance in rats. The more rapidly glucose was absorbed from the diet, the faster the insulin resistance developed.\(^{17}\) Insulin resistance is considered to be one of the main determinants of the hyperglycemia, hyperinsulinemia, and impaired glucose tolerance in NIDDM.\(^{18}\) FA is more viscous than cellulose when dissolved in water (data not shown). When FA was added to the basal diet to replace cellulose, the increased viscosity of the gastrointestinal contents probably reduced the rate of emptying of the stomach, altered motility in the stomach and small intestine, and reduced the rate of carbohydrate absorption. Consequently, the delayed carbohydrate absorption due to FA administration may be an important factor to improve the hyperglycemia, hyperinsulinemia, severe insulin resistance, and impaired glucose tolerance of KK-A' mice. This assumption is supported by the fact that the postprandial serum glucose and insulin concentrations were lower in the mice fed on the FA diet than in the mice fed on the control diet in the final diet administration test.

The restriction of dietary intake by the food-restricted group slightly lowered the fasting plasma glucose and urinary glucose levels. One of the possible reasons for this is the reduced carbohydrate intake. However, food restriction with the control diet failed to improve the postprandial hyperglycemia and hyperinsulinemia, as well as the glucose tolerance of the KK-A' mice. The reasons for this are probably that the food restriction did not delay the rate of carbohydrate absorption in the small intestine and reduce the insulin resistance of KK-A' mice.

Diabetes is known to impair the normal capacity of the liver to synthesize glycogen in streptozotocin-induced or alloxan-induced diabetic rats.\(^{19-21}\) The present study found that an FA intake significantly increased the hepatic glycogen content in KK-A' mice. This may be considered to be a contributing factor to lower the postprandial serum glucose concentration, reduce insulin secretion, and increase glucose tolerance. Certainly, this notion needs to be further investigated.

The hypoglycemic effect of dietary fiber on diabetes has often been considered to depend on its consumption in a large quantity.\(^{22,23}\) However, in this study, the total amount of the dietary fiber in the FA diet was kept at the same level as that in the basal diet. When cellulose in the basal diet, an insoluble polysaccharide which has been indicated to have no hypoglycemic effect on KK-A' mice,\(^{24}\) was partially replaced by FA, a significant hypoglycemic effect was observed on KK-A' mice. The present results suggest for the dietary management of diabetes that increasing the proportion of a water-soluble dietary fiber that has been confirmed to be effective for improving diabetic symptoms in the total dietary fiber intake might be more beneficial than only significantly increasing the dietary fiber consumption or only restricting the food intake.

Sone et al. have studied the chemical constitution of the polysaccharides from \textit{A. auricula-judae} and reported that the water-soluble polysaccharides of \textit{A. auricula-judae} contained a large amount of acidic heteropolysaccharide and a small amount of neutral \(\beta\)-d-glucan.\(^{25}\) The water-soluble crude polysaccharide used in this study is considered to have been a mixture of the acidic and neutral polysaccharides. At present, it is not known which water-soluble polysaccharide from \textit{A. auricula-judae}, the acidic or neutral polysaccharide, mainly contributed to the hypoglycemic action of FA. Further studies are now in progress to clarify this.

In conclusion, this study has shown that the water-soluble polysaccharide from \textit{A. auricula-judae} had a hypoglycemic effect on KK-A' mice. This study has also confirmed that the inhibitory effect of this extract on food consumption was not a major factor to cause the hypoglycemic action on diabetic animals.

References
Hypoglycemic Effect of Auricularia auricula-judae Quel.


