— **Regular Article** —

Dietary *Sparassis crispa* (Hanabiratake) Ameliorates Plasma Levels of Adiponectin and Glucose in Type 2 Diabetic Mice

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Sparassis crispa (SC), known as Hanabiratake in Japanese, is an edible mushroom with medicinal properties; however, its antidiabetic activity is not well established. In the present study, we examined the effects of dietary SC on diabetic mice. KK-Ay mice that were fed SC for 3 or 6 weeks showed pronounced increase of plasma levels of adiponectin. Significant decrease of blood glucose and insulin levels were also observed by 3 week-administration of SC. Moreover, mice that were fed the SC diet exhibited relatively decreased serum levels of triglycerides and total cholesterol. Although the SC diet had no effect on body and adipose tissue weights in KK-Ay mice, the size of the mesenteric adipose cells of SC group was smaller than control group though it was not significant difference. Thus, the SC diet might decrease the adipose cell size in order to increase plasma adiponectin levels. Considering the physiological significance of adiponectin in type 2 diabetes, insulin resistance, and cardiovascular diseases, these findings imply that dietary SC has the potential to ameliorate these diseases.

Key words —— *Sparassis crispa* (Hanabiratake), adiponectin, KK-Ay mice

INTRODUCTION

Research efforts have been increasingly directed towards medical treatment and prevention of metabolic syndrome, such as obesity, type 2 diabetes, and cardiovascular diseases. Type 2 diabetes, a metabolic syndrome disease characterized by hyperglycemia and dyslipidemia resulting from defects in both insulin secretion and insulin sensitivity, has become a significant and growing problem in both developed and developing countries.

It is well known that the adipose tissue is not only a storage depot for fat but also functions as an endocrine organ and plays a key role in the control of endocrine signaling, glucose metabolism, inflammation, and energy homeostasis.[1)] These functions of the adipose tissue are mediated through a number of adipocytokines[1–3)] of which adiponectin has been established as a key adipocytokine in the regulation of metabolic syndrome.[4,5)] Adiponectin also plays a pivotal role in the improvement of insulin sensitivity, as well as in the amelioration of glycemic control and type 2 diabetes.[1,5,6)]

Although there has been considerable progress in the management of diabetes mellitus by synthetic drugs, there is an increasing demand for natural products with antidiabetic activity because of the side effects associated with the use of insulin and synthetic drugs. It has been previously reported that some mushrooms such as *Lyophyllum decastes*,[7)] *Grifola frondosa*,[8)] and *Agaricus blazei*[9)] exhibit antidiabetic activities. However, the effect of mushrooms on glycemic responses and plasma adiponectin levels remains mostly unclear.

Sparassis crispa (SC), known as Hanabiratake in Japanese, is an edible mushroom with various medicinal properties that has recently become cultivable in Japan. SC primarily grows on the stumps of coniferous trees and is widespread in northern temperate zones worldwide.[10)] More than 40% of SC consists of β-D-glucan, which is comprised of a β-(1→3)-D-glucan backbone with a single β-(1→6) or β-(1→2)-D-glucosyl side-branching unit occurring every three or four residues.[11–13)] SC has been reported to have many biological activities such as tumor-suppressing effects,[11,13,14)] improve-

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ment in natural killer cell activity,[14] antiangiogenic effects,[13] antiallergic effects,[15] wound healing effects,[16] and enhancements in hematopoietic responses.[11,17] However, no studies have been performed to elucidate the antidiabetic properties of SC. In the present study, we examined the beneficial effects of SC on glycemic responses and plasma levels of adiponectin and insulin in obese type 2 diabetic mice.

MATERIALS AND METHODS

Fungus —— The fruit bodies of SC cultivated by Unitika Ltd. (Aichi, Japan) were used in the present experiment. These fruit bodies were freeze-dried and ground into an ultrafine power by a mill. The average diameter of each powder grain was 8 μm.

Animals —— Four-week old male KK-Ay mice (Clea Japan, Osaka, Japan) were used in the present study. The mice were housed in an air-conditioned room at 22 ± 2°C with a 12 hr light-dark cycle (light: 7:00 a.m. to 7:00 p.m.). They were kept in an experimental animal room for 7 days with free access to food (CE-2; Clea Japan) and water (tap water) and divided into two groups: the control group, which were fed a diet containing 1.2% cellulose (KC flock; Nippon Paper Chemicals Co., Ltd., Tokyo, Japan), and the SC group, which was fed a diet containing 5.0% SC. The total dietary fiber content of each diet was 5.2% and 6.7%, respectively. The mice were fed their assigned diets for 7 weeks. All mice were cared for in accordance with the Guideline for the Care and Use of Laboratory Animals (the Prime Minister's Office No.6, 1980).

Determination of Blood Glucose, Plasma Insulin C-peptide, and Adiponectin Levels —— Blood glucose concentrations were monitored once a week during the experimental period. And fasting blood glucose was also measured at 3 and 6 week of experiment using a glucometer (Glucocard G+ meter; Arkray Co. Ltd., Kyoto, Japan). For biochemical examination of Insulin C-peptide II and adiponectin at 3 and 6 weeks, blood sample were taken from tail vein and collected plasma by immediate centrifugation (3000 rpm, 5 min, 4°C). Insulin C-peptide is released by processing of pro-insulin to matured-insulin and its levels are often measured instead of insulin levels clinically, because it has a longer half-life than insulin. Plasma insulin C-peptide II was measured using a mouse insulin C-peptide II EIA Kit (Yanaihara Institute Inc., Shizuoka, Japan). Adiponectin concentrations were measured using a mouse adiponectin/Acrp30 EIA Kit (R&D systems, Inc., Minneapolis, MN, U.S.A.).

Measurement of Serum Total Cholesterol (T-Cho), Triglycerides (TG), and Cell Size of Adipose Tissue —— After receiving their assigned diets for 7 weeks, each mouse was sacrificed by collection of whole blood under pentobarbital anesthesia (50 mg/kg). Serum samples were collected and intra-abdominal adipose tissues (mesenteric, epididymal, and kidney leaf fat tissues) were excised and weighed. Serum T-Cho and TG levels in all mice were determined using the Cholesterol E-test and Triglyceride E-test (Wako, Osaka, Japan). Mesenteric fat tissues of the control and SC groups were stained using hematoxylin-eosin after fixing with 20% neutral buffered formalin (Wako). The area of the adipose tissue cells were measured using image processing software (imageJ version 1.42 q, National Institutes of Health, Bethesda, MD, U.S.A.) under a high-power microscopic field (magnification × 100).

Statistical Analysis —— All data were represented as mean ± S.E. Differences among means were analyzed by the Student's *t*-test or two-way analysis of variance (ANOVA). Differences were considered significant at $p < 0.05$.

RESULTS

Effects of SC on Blood Glucose and Insulin C-peptide II level, and Body Weight in KK-Ay Mice

The measured blood glucose levels are shown in Fig. 1. SC-fed KK-Ay mice maintained lower constant blood glucose levels compared to the control mice during the experimental period. More-

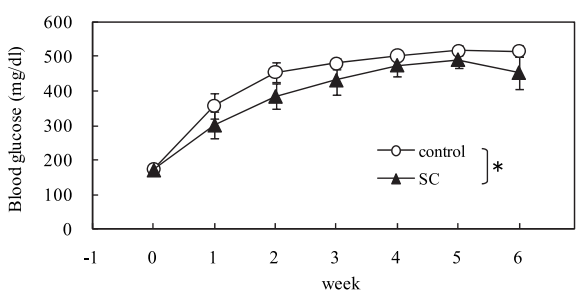

Fig. 1. Blood Glucose Level Profiles during the Experimental Period for KK-Ay Mice

Each value represents the mean ± S.E. of 6–8 mice. * $p < 0.05$ (*vs.* control group, two-way ANOVA).

over, SC-fed KK-Ay mice showed reduced fasting blood glucose and fasting plasma insulin levels, especially significantly at 3 weeks (Fig. 2). The temporal changes in body weight are shown in Fig. 3. Even after 6 weeks of repeated administration of SC, no significant differences in body weight was observed between KK-Ay mice assigned to either the SC or control group.

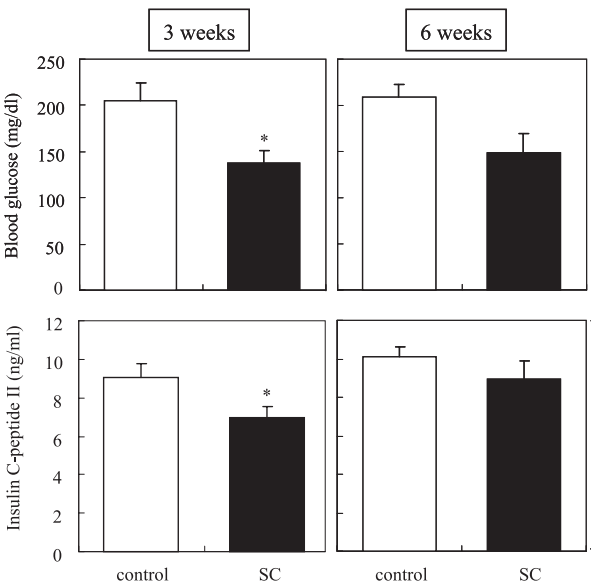

Fig. 2. Fasting Blood Glucose and Plasma Insulin C-peptide II Levels of KK-Ay Mice 3 and 6 Weeks after Administration of the Experimental Diet

Each value represents the mean ± S.E. of 6–8 mice. $^{*}p < 0.05$ (*vs.* control group).

Effects of SC on Serum TG and T-Cho Levels of KK-Ay Mice

The serum TG and T-Cho levels in KK-Ay mice 7 weeks after administration are shown in Fig. 4. These levels were relatively lower in the SC group than in the control group.

Effects of SC on Plasma Adiponectin Levels of KK-Ay Mice

The plasma adiponectin levels in SC-fed KK-Ay mice 3 and 6 weeks after administration are shown in Fig. 5. Plasma adiponectin concentrations were notably higher in the SC group than in the control group.

Tissue Weight and Cell Size of Intra-abdominal Adipose Tissues

The weights and cell size of intra-abdominal adipose tissues are shown in Table 1, and picture

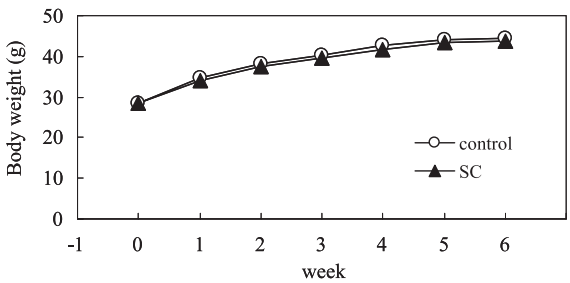

Fig. 3. Temporal Changes in Body Weight during the Experimental Period of KK-Ay Mice

Each value represents the mean ± S.E. of 6–8 mice.

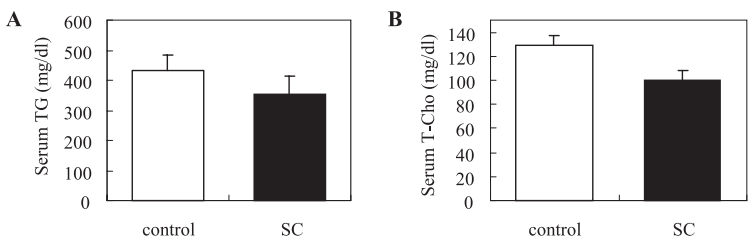

Fig. 4. Serum TG (A) and T-Cho (B) Levels in KK-Ay Mice 7 Weeks after Administration of the Experimental Diet

Each value represents the mean ± S.E. of 6–8 mice.

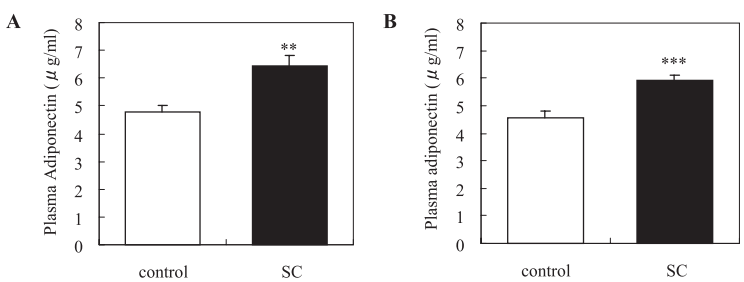

Fig. 5. Plasma Adiponectin Levels in KK-Ay Mice (A) 3 Weeks and (B) 6 Weeks after Administration of the Experimental Diet

Each value represents the mean ± S.E. of 7–8 mice. $^{**}p < 0.01$; $^{***}p < 0.001$ (*vs.* control group).

Table 1. Weights of Intra-abdominal Adipose Tissues and Cell Sizes of Mesenteric Adipose Tissue

group	adipose tissue weight (g)			cell area
	mesenteric	epididymal	kidney leaf	(% *vs.* control)
control	1.13 ± 0.08	1.61 ± 0.04	0.85 ± 0.07	100.0 ± 15.0
SC	1.14 ± 0.06	1.67 ± 0.11	0.90 ± 0.07	75.4 ± 10.3

Each value represents the mean ± S.E. of 4–8 mice.

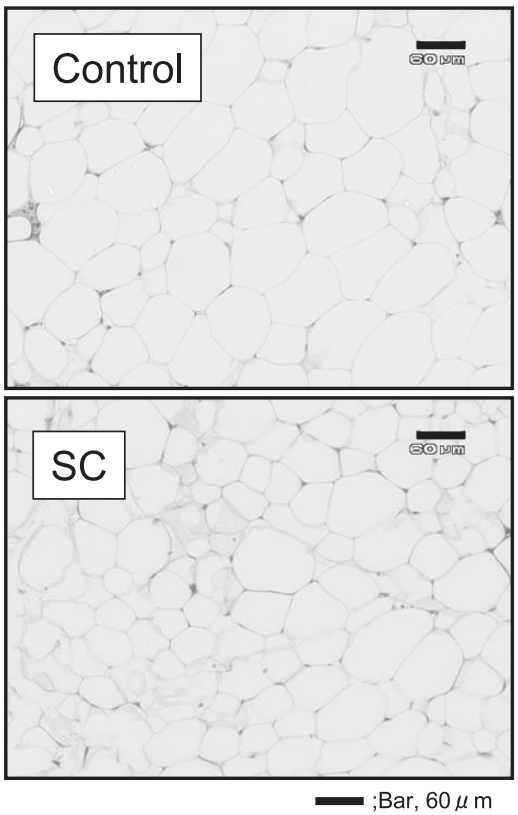

Fig. 6. Representative Microscope Photographs of Mesenteric Adipose Tissues of Control Group and SC Group

of mesenteric abdominal cells representing each group was appeared in Fig. 6. Although there were no marked differences in the adipose tissue weight among the dietary groups, the mean of the mesenteric adipose cells size was about 25% smaller in the SC group than in the control group.

DISCUSSION

In the present study, we observed that the SC-fed KK-Ay mice, an animal model of type 2 diabetes mellitus, exhibited significantly increased plasma levels of adiponectin at 3 and 6 week administration of SC (Fig. 5) as well as a significantly decreased blood glucose and insulin levels at 3 week (Figs. 1 and 2) compared to KK-Ay mice fed with a control diet. Moreover, the SC diet decreased the serum TG and T-Cho levels, but not significant differences (Fig. 4). Increase in the level of adiponectin as well as the decrease in the levels of blood glucose and insulin are of particular interest, as there are only a few reports indicating that dietary mushrooms can have beneficial effects on the plasma levels of adiponectin. It is well established that increased adiponectin levels stimulate glucose utilization through the activation of adenosine monophosphate (AMP)-activated protein kinases in skeletal muscle and liver.[3] Thus, administered-SC should reduce glucose levels due to improved incorporation of glucose into peripheral tissues in response to SC-induced elevated adiponectin levels. Mice lacking adiponectin exhibit severe diet-induced insulin resistance.[18] Furthermore, adiponectin has been found to reverse insulin resistance associated with both lipoatrophy and obesity. Hence, it is suggested that adiponectin is a potent insulin enhancer, linking adipose tissue and whole-body glucose metabolism.[19] The findings of this study strongly support the possibility that dietary SC could enhance insulin action through its effect on adiponectin, and suggest that SC has the potential to ameliorate or attenuate insulin resistance in type 2 diabetes accompanied by hyperlipidemia.

The size of adipocytes influences the gene expression and secretions of adipocytokines such as adiponectin and tumor necrosis factor-α (TNF-α).[20] No effects on body and adipose tissue weights were observed in SC-fed KK-Ay mice (Fig. 3 and Table 1). However, the size of the mesenteric adipose cells was relatively smaller in the SC group than in the control group (Table 1 and Fig. 6). Therefore, it is likely that SC feeding might decrease the adipose cell size in response to increased plasma adiponectin levels.

On the contrary, it is well established that treatment with thiazolidinediones or insulin-sensitizing agents increases adiponectin levels through increased peroxisome proliferator-activated receptor-γ (PPAR-γ) activity.[21,22] Therefore, future studies

should address the question of whether SC feeding modulates PPAR-γ activity.

An epidemiological study has reported an interesting observation that a diet low in glycemic load and high in fiber, obtained from dietary cereal, increases adiponectin levels in diabetic men.[23] Thus, it is likely that a specific kind of fiber in SC, such as β-(1→3)-D-glucan, contributed to the observed increase in adiponectin levels. It is very interesting that the antidiabetic effects of this fiber in SC are higher than that of cellulose.

In conclusion, this study clarifies the beneficial effects of dietary SC on the levels of adiponectin, glucose and insulin. The toxicity of SC seems to be very low (LD_{50} > 5000 mg/kg body weight). Moreover, SC-treated (5000 mg/kg) mice did not show any obviously harmful action (data not shown). Considering the physiological significance of adiponectin in type 2 diabetes, insulin resistance, and cardiovascular disease, the present findings suggest that dietary SC has the potential to safely ameliorate these diseases.

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