Dietary *Sparassis crispa* Reduces Body Fat Mass and Hepatic Lipid Levels by Enhancing Energy Expenditure and Suppressing Lipogenesis in Rats

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**Abstract:** Accumulation of abdominal fat triggers metabolic syndrome, which is a cluster of metabolic abnormalities, such as dyslipidemia, glucose intolerance, insulin resistance or hyperinsulinemia, and hypertension, that leads to the development of diabetes and cardiovascular disease. Mushrooms have been used as a foodstuff and folk medicine worldwide. Among these mushrooms, *Sparassis crispa* (SC) is a relatively newly cultivated edible and medicinal mushroom, which has been reported to have anti-diabetic and anti-hypertensive properties. However, little is known about the anti-obesity and anti-hyperlipidemic properties of SC. In the present study, we investigated the effects of dietary SC on lipid metabolism and energy expenditure in Sprague-Dawley rats with diet-induced obesity and diabetes, and conducted respiratory gas analysis to determine how energy metabolism is altered by SC. After feeding periods of 3 and 7 weeks, dietary SC had significantly reduced hepatic triacylglycerol and cholesterol contents in a dose-dependent manner. These changes were attributable to suppression of fatty acid and cholesterol synthesis in the liver and increased insulin sensitivity in the body. In addition, after a feeding period of 6 weeks, dietary SC significantly increased energy expenditure through carbohydrate oxidation, reducing abdominal fat mass after 7 weeks. In conclusion, our results indicate that in addition to the previously reported anti-diabetic and anti-hypertensive activities, dietary SC exhibits anti-obesity and anti-hyperlipidemic activities that help protect against metabolic syndrome.

**Key words:** *Sparassis crispa*, body fat mass, hepatic lipid accumulation, energy expenditure

1 Introduction

Metabolic syndrome is a cluster of metabolic abnormalities, such as abdominal fat accumulation, dyslipidemia, glucose intolerance, insulin resistance or hyperinsulinemia, and hypertension that leads to the development of diabetes and cardiovascular disease.² Because the accumulation of abdominal fat can trigger metabolic syndrome, it is critical to attain and maintain a healthy bodyweight. Abdominal and subcutaneous fats are the two types of fat in the body. Abdominal fat is mainly derived from dietary fat or excess energy intake due to an imbalance between energy intake and energy expenditure. Therefore, in order to combat the obesity epidemic, it is important to reduce energy intake or increase energy expenditure. To this end, several food ingredients have been extensively studied and reported to have anti-obesity activity through enhancement of energy expenditure, including 10*trans*,12*cis*-conjugated linoleic acid⁶, resveratrol⁶, pterostilbene⁶, fish oil³, the probiotic *Lactobacillus gasseri* SBT2055⁶, and *Agaricus*⁷.

Mushrooms have been used as foodstuffs and folk medicines globally. Several studies have aimed to identify the active ingredients in mushrooms, which have anti-tumor,
anti-inflammatory, and anti-cardiovascular disease effects. For example, when obese mice were administered maitake mushroom (Panellus serotinus), there was a significant reduction in hepatic lipids, partly due to a decrease in hepatic lipogenic enzyme activity and enhancement in carnitine palmitoyltransferase activity, the latter of which is responsible for fatty acid oxidation. Enokitake (Flammulina velutipes) has been shown to alleviate hyperlipidemia, likely through enhanced gene expression of hepatic low-density lipoprotein receptor in rats. Feeding rats a diet of 4% shiitake mushroom (Lentinus edodes) significantly stimulated energy expenditure. Collectively, it is hypothesized that mushrooms exert their anti-obesity activity by downregulating lipogenesis and/or enhancing energy expenditure. Sparassis crispa (SC), also known as cauliflower mushroom, grows near the bases of conifer trunks and stumps and is widely distributed in Northern temperate zones worldwide. SC is a relatively newly cultivated edible and medical mushroom, and has been reported to have anti-diabetic and anti-hypertensive properties. However, little is known about the anti-obesity and anti-hyperlipidemic activities of SC.

In the present study, because the ability of SC to decrease lipid levels has not been thoroughly studied, and enhanced energy expenditure is an effective anti-obesity therapy, we focused on determining how SC affects lipid metabolism and energy expenditure. The present study consisted of two experiments. In the first experiment, Sprague-Dawley (SD) rats were fed a diet consisting of 2 or 4% SC for 3 weeks and lipid parameters were measured. In the second experiment, 2 and 4% SC diets were administered to SD rats for 7 weeks and lipid parameters were analyzed. In addition, we conducted respiratory gas analysis to determine how energy metabolism is altered by SC.

2 Experimental

2.1 Materials

SC was provided by Chukoh Chemical Industries, Ltd. (Nagasaki, Japan). SC was sliced, lyophilized for 2 days using a freeze dryer (EYELA FD-550R, Tokyo Rikakikai Co., Ltd., Tokyo, Japan), and powdered. The chemical composition of the SC powder was analyzed by the Institute of Food Hygiene, Nagasaki Food Hygiene Association, and Food and Environment Research Center (Nagasaki, Japan), and determined to be as follows: 55.9% carbohydrate (including dietary fiber), 29.2% protein, 5.8% ash, and 2.1% fat.

2.2 Animals and diets

Male Sprague-Dawley rats (Jcl: SD) were obtained from CLEA Japan, Inc. (Osaka, Japan). Rats were housed individually in metal cages in a temperature-controlled room (22°C ± 1°C) under a 12 h light-12 h dark cycle. After a 9-day adaptation period, rats were assigned to one of the three groups. The control group was fed a modified AIN-93G diet. For rats that were fed the experimental diets, SC powder was added to the control diet to a final concentration of 2 or 4%. The compositions of the diets are presented in Table 1. The diets contained high fructose and lard rich in saturated fatty acid to induce obesity and insulin resistance.

In Experiment 1, 6-week-old male SD rats were fed ad libitum for 3 weeks (n = 6/group). After 3 weeks, the rats were fasted for 6 h and then sacrificed by decapitation. The liver, cecum, and white adipose tissue (WAT, epididymal, perirenal and mesenteric) were excised immediately.

To obtain serum, blood was collected, incubated at room temperature for 30 min, and centrifuged at 1,200 × g for 15 min at 4°C. Samples were stored at −80°C until analysis.

In Experiment 2, 5-week-old male SD rats were fed ad libitum for 7 weeks (n = 8/group). Rats were subjected to respiratory gas analysis after 6 weeks of feeding the experimental diet. At the end of the feeding period, the rats were sacrificed by decapitation without prior fasting. The liver, cecum, and WAT (epididymal, perirenal and mesenteric) were excised immediately. Serum was collected as described above. Samples were stored at −80°C until analysis.

All aspects of this study were conducted in accordance with the Guidelines for Animal Experiments of University

### Table 1 Composition of experimental diets.

| Ingredients              | Control | SC 2%  | SC 4%  |
|--------------------------|---------|--------|--------|
| Casein (%)               | 20.0    | 20.0   | 20.0   |
| α-Corn starch (%)        | 13.2    | 13.2   | 13.2   |
| Sucrose (%)              | 10.0    | 10.0   | 10.0   |
| Fructose (%)             | 20.0    | 20.0   | 20.0   |
| Cellulose (%)            | 5.00    | 5.00   | 5.00   |
| Mineral mix (AIN-93G) (%)| 3.50    | 3.50   | 3.50   |
| Vitamin mix (AIN-93) (%) | 1.00    | 1.00   | 1.00   |
| l-Butylhydroquinone (%)  | 0.0014  | 0.0014 | 0.0014 |
| Choline bitartrate (%)   | 0.250   | 0.250  | 0.250  |
| l-Cystine (%)            | 0.300   | 0.300  | 0.300  |
| Soybean oil (%)          | 1.00    | 1.00   | 1.00   |
| Lard (%)                 | 14.0    | 14.0   | 14.0   |
| Sparassis crispa (SC) (%)| –       | 2.00   | 4.00   |
| β-Corn starch (%)        | 11.586  | 9.586  | 7.586  |
| Calorie (kcal/g)         | 4.30    | 4.26   | 4.23   |
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of Nagasaki, Siebold and Law no. 105 and Notification no. 6 of the government of Japan. The animal protocols in the present study were approved by the Institutional Review Board of University of Nagasaki, Siebold (authorization no. 27-21 and 28-03).

2.3 Respiratory gas analysis

After 6 weeks of being fed the experimental diets, each rat was placed in a metabolic chamber (210 × 220 × 370 mm) for 24 h to measure VO$_2$ (oxygen exhaustion) and VCO$_2$ (carbon dioxide emission). During respiratory gas analysis, rats had free access to food and water. The system consisted of twelve acrylic metabolic chambers, a mass spectrometer (model ARCO-2000), gas sampler (model ARCO-2000-GS-12), and software (model ARCO-2000-RAT). All instrumentation and software was obtained from Arco System (Chiba, Japan). Room air was pumped through the chambers at a rate of 1.0 L/min. Expired air was directed to a mass spectrometer. Air from each chamber was sampled every 5 min and the resulting data was recorded in a spreadsheet. Carbohydrate and fat oxidation, and energy expenditure were calculated using the following formulas:

\[
\text{Carbohydrate oxidation} = 4.51 \times V_{\text{CO}_2} - 3.18 \times V_{\text{O}_2}
\]

\[
\text{Fat oxidation} = 1.67 \times (V_{\text{O}_2} - V_{\text{CO}_2})
\]

\[
\text{Energy expenditure} = 3.816 \times V_{\text{O}_2} + 1.231 \times V_{\text{CO}_2}
\]

2.4 Measurement of serum and liver parameters

Serum levels of glucose, triacylglycerol (TAG), cholesterol, phosphatidylcholine (PC), and free fatty acid were measured using commercial enzyme assay kits (Glucose CII-Test, Triacylglycerol E-Test, Cholesterol E-Test, Phospholipid C-test, and NEFA C-Test, respectively; Wako, Osaka, Japan). Serum insulin levels were measured using a commercial enzyme-linked immunosorbent assay kit (Ultra Sensitive Rat Insulin ELISA Kit, Morinaga Institute of Biological Science, Inc., Kanagawa, Japan).

Liver lipids were extracted according to a method previously described by Folch et al.\(^\text{[10]}\), and hepatic TAG, cholesterol, and PC levels were determined using the aforementioned commercial enzyme assay kits. Liver glycogen was extracted and measured using an anthrone-sulfuric acid-based method published by Lo S et al.\(^\text{[17]}\).

2.5 Preparation of hepatic subcellular fractions

An aliquot of liver was homogenized in 6 volumes of a 0.25 M sucrose solution containing 1 mM EDTA in 10 mM Tris-HCl buffer (pH 7.4). After the nuclear fraction was precipitated, the supernatant was centrifuged at 10,000 × g for 10 min at 4°C to separate out the mitochondrial frac-
tion. This supernatant was centrifuged at 125,000 × g for 60 min at 4°C to precipitate out the microsomal fraction and the resulting supernatant was considered the cytosolic fraction. Protein concentrations were measured as described by Lowry et al.\(^\text{[18]}\) using bovine serum albumin as the standard.

2.6 Measurement of liver enzyme activity

Hepatic lipid metabolism-related enzyme activity was measured to determine how SC alters TAG metabolism. The enzymatic activity of malic enzyme\(^\text{[19]}\), glucose-6-phosphate dehydrogenase\(^\text{[20]}\), and fatty acid synthase\(^\text{[21]}\) in the hepatic cytosolic fraction and phosphatidate phosphohydrolase\(^\text{[22]}\) in the hepatic microsomal fraction was measured.

2.7 Analysis of mRNA expression

Cholesterol metabolism-related mRNA expression was measured in Experiment 2. Total RNA was extracted from 100 mg of liver using RNAiso Plus (Takara Bio Inc., Shiga, Japan) and then prepared to be template RNA using DNAse I (Toyobo, Osaka, Japan) and a real-time PCR system (LightCycler 96 System; Roche Diagnostics Co., Ltd, Tokyo, Japan). Primer sequences used were as follows: 3-hydroxy-3-methyl-gulataeryl-CoA reductase (HMG-CoA reductase; GenBank accession no. NM_013134.2), forward primer, 5'-ACTCATTCTCTACCTC-3' and reverse primer, 5'-AGGCCCTTTGAAACCTA-3'; sterol regulatory element binding transcription factor 2 (SREBP2; GenBank accession no. NM_001033694.1) forward primer, 5'-AGCATACGCAAGGTTCCCTCG-3' and reverse primer, 5'-CCAGGTGTCTTACTTCTCCGTG-3'; and β-actin (GenBank accession no. NM_031144.3) forward primer, 5'-ACTCTGTGTGATGATTGGTGACC-3' and reverse primer, 5'-TCATCGTACTCTCATCCTTGTCCTG-3'. Results were quantified using a comparative method and expressed as relative values after normalization to β-actin.

2.8 Analysis of fecal lipids

In Experiment 2, feces were collected for 2 days at the end of the study to quantify lipid excretion. Collected feces were lyophilized for 2 days and then ground. Fecal neutral steroids were analyzed by a gas chromatograph equipped with a SPB-1 fused silica capillary column (0.25 mm × 30 m, 0.25 μm thickness, Sigma-Aldrich Japan, Tokyo, Japan) using 5a-cholestane as an internal standard as described previously\(^\text{[23]}\) with slight modifications. Neutral steroid levels were calculated based on the sum of the amounts of cholesterol and coprostanol. Fecal fatty acid excretion was measured by titration according to the method described in the text.
2.9 Statistical analysis
All values are expressed as mean ± standard error of mean. Comparisons of the groups were performed using one-way ANOVA followed by the Williams’ test. Differences were considered statistically significant at $p < 0.05$. Statistical analyses were performed using Excel 2016 (Microsoft, USA) with the add-in software Excel-Toukei 2010 (Social Survey Research Information Co., Ltd., Tokyo, Japan).

3 Results
3.1 Effects of Short-Term Feeding of SC on Growth and Metabolic Parameters (Experiment 1)
Table 2 summarizes the growth and metabolic parameters of rats fed the indicated diets for 3 weeks and then sacrificed following fasting. There were no significant differences in the final body weight, calorie intake, WAT weights, cecum weight, and serum parameters between the groups. Conversely, the SC 4% group had a significantly lower liver weight than the control group. Diets containing SC markedly decreased hepatic levels of TAG, cholesterol, and PC in a dose-dependent manner.

3.2 Effects of Long-Term Feeding of SC on Growth and Metabolic Parameters (Experiment 2)
Table 3 lists the growth and metabolic parameters of experimental rats after a 7-week feeding period. Since differences in parameters would be expected to be greater when the rats were not fasted, the rats were sacrificed without prior fasting. No significant difference in final body weight and calorie intake was observed between the groups. The SC 4% diet significantly decreased the liver and perirenal WAT weights, but increased the cecum weight relative to

| Table 2 | Effects of short-term feeding of SC on growth and metabolic parameters in rats (Experiment 1). |
|---------|--------------------------------------------------------------------------------------------------|
|         | Control | SC 2% | SC 4% |
| Initial body weight (g) | 179 ± 3 | 179 ± 3 | 176 ± 3 |
| Final body weight (g) | 348 ± 11 | 346 ± 9 | 322 ± 5 |
| Food intake (g/day) | 21.6 ± 0.9 | 22.1 ± 0.9 | 20.3 ± 0.6 |
| Calorie intake (kcal/day) | 93.1 ± 3.8 | 94.1 ± 3.9 | 85.8 ± 2.5 |
| Liver weight (g) | 16.3 ± 1.0 | 15.6 ± 0.8 | 12.8 ± 0.4† |
| Cecum weight without content (g) | 0.575 ± 0.041 | 0.617 ± 0.063 | 0.582 ± 0.046 |
| White adipose tissue (g) | | | |
| Epididymal | 4.42 ± 0.52 | 4.47 ± 0.32 | 3.31 ± 0.36 |
| Perirenal | 4.81 ± 0.88 | 5.74 ± 0.71 | 2.97 ± 0.52 |
| Mesenteric | 3.47 ± 0.46 | 4.20 ± 0.59 | 2.66 ± 0.24 |
| Total abdominal | 12.7 ± 1.8 | 14.4 ± 1.5 | 8.9 ± 1.1 |
| Serum parameters | | | |
| Glucose (mg/dL) | 121 ± 5 | 114 ± 4 | 109 ± 1 |
| Triacylglycerol (mg/dL) | 186 ± 62 | 179 ± 27 | 105 ± 9 |
| Cholesterol (mg/dL) | 94.4 ± 6.1 | 90.3 ± 5.2 | 78.8 ± 4.3 |
| Phosphatidylcholine (mg/dL) | 137 ± 12 | 137 ± 7 | 115 ± 5 |
| Free fatty acid (mEq/L) | 0.910 ± 0.115 | 0.926 ± 0.039 | 0.810 ± 0.025 |
| Liver lipids (mg/whole liver) | | | |
| Triacylglycerol | 1374 ± 174 | 1121 ± 230 | 576 ± 100† |
| Cholesterol | 354 ± 22 | 267 ± 50 | 161 ± 15† |
| Phosphatidylcholine | 276 ± 13 | 237 ± 11* | 194 ± 22† |

Table values are presented as mean ± standard error of the mean (SEM), where $n = 6$ / group. *, Significantly different from control ($p < 0.05$) †, Significantly different from control ($p < 0.05$)
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The SC diet did not alter serum levels of glucose and lipids. However, serum insulin levels were markedly lower in the SC 2 and 4% groups than in the control group. In accordance with the results observed for the short-term feeding (Experiment 1), the SC diet led to significantly lower hepatic lipid levels in a dose-dependent manner. Liver glycogen levels remained unaltered by these diets.

3.3 Effects of Long-Term Feeding of SC on Nutrient Oxidation and Energy Expenditure (Experiment 2)

The effect of the SC diet on energy metabolism was assessed using respiratory gas analysis of rats fed a similar number of calories of the control, SC 2%, or SC 4% diet (control group, 85.9 ± 5.5 kcal/day; SC 2% group, 91.8 ± 5.5 kcal/day; SC 4% group, 96.2 ± 4.9 kcal/day).

As shown in Fig. 1, the SC diets significantly enhanced carbohydrate oxidation during the second half of the light cycle and suppressed fat oxidation during the light cycle, resulting in a shift in the carbohydrate and fat oxidation ratio. Energy expenditure was temporarily enhanced by the SC diet around 20:00.

3.4 Effects of Long-Term Feeding of SC on Metabolic Parameters Related to Lipid Metabolism in the Liver (Experiment 2)

A decrease in hepatic TAG and cholesterol was observed; therefore, hepatic lipid synthesis and gene expression were evaluated to identify the underlying mechanisms. Table 4 summarizes parameters related to lipid metabolism in rats after 7 weeks of feeding. Regarding hepatic TAG synthesis, phosphatidate phosphohydrolase activity remained unchanged, whereas fatty acid synthase activity in the SC 4% group decreased by 40% ($p < 0.05$) compared to that in the control group. Malic enzyme and glucose-6-phosphate dehydrogenase activity in the livers of non-fasted rats was decreased.

### Table 3 Effects of long-term feeding of SC on growth and metabolic parameters in rats (Experiment 2)

| Parameter                              | Control | SC 2% | SC 4% |
|----------------------------------------|---------|-------|-------|
| Initial body weight (g)                | 152 ± 3 | 152 ± 2 | 152 ± 2 |
| Final body weight (g)                  | 539 ± 14 | 512 ± 12 | 507 ± 12 |
| Food intake (g/day)                    | 23.2 ± 0.8 | 22.1 ± 0.5 | 21.4 ± 0.5 |
| Calorie intake (kcal/day)              | 99.6 ± 3.5 | 94.1 ± 2.1 | 90.4 ± 2.0 |
| Liver weight (g)                       | 26.9 ± 1.3 | 24.2 ± 0.8 | 22.8 ± 0.8† |
| Cecum weight without content (g)       | 0.536 ± 0.020 | 0.538 ± 0.016 | 0.860 ± 0.117† |
| White adipose tissue (g)               |         |       |       |
| Epididymal                             | 11.7 ± 1.1 | 10.6 ± 0.7 | 9.9 ± 0.7 |
| Perirenal                              | 18.5 ± 1.6 | 16.5 ± 0.7 | 13.4 ± 1.2† |
| Mesenteric                             | 7.66 ± 0.73 | 6.65 ± 0.41 | 5.76 ± 0.53 |
| Total abdominal                        | 37.8 ± 3.2 | 33.8 ± 1.5 | 29.0 ± 2.3 |
| Serum parameters                       |         |       |       |
| Glucose (mg/dL)                        | 123 ± 2 | 124 ± 4 | 115 ± 5 |
| Triacylglycerol (mg/dL)                | 678 ± 93 | 697 ± 85 | 562 ± 83 |
| Cholesterol (mg/dL)                    | 136 ± 5 | 127 ± 8 | 135 ± 11 |
| Phosphatidylcholine (mg/dL)            | 243 ± 19 | 229 ± 12 | 216 ± 16 |
| Free fatty acid (mEq/L)                | 1.39 ± 0.14 | 1.55 ± 0.18 | 1.33 ± 0.15 |
| Insulin (ng/mL)                        | 3.73 ± 0.38 | 1.97 ± 0.26* | 1.78 ± 0.28† |
| Liver lipids (mg/whole liver)          |         |       |       |
| Triacylglycerol                        | 2908 ± 353 | 2440 ± 220 | 1876 ± 204† |
| Cholesterol                            | 636 ± 46 | 570 ± 50 | 404 ± 39† |
| Phosphatidylcholine                    | 571 ± 44 | 469 ± 20* | 391 ± 22† |
| Liver glycogen (mg/whole liver)        | 243 ± 17 | 221 ± 22 | 223 ± 20 |

Values are presented as mean ± SEM ($n$ = 6-8/group).

*, Significantly different from control ($p < 0.05$)

†, Significantly different from control ($p < 0.05$)

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significantly suppressed by the SC diets. The SC 4% diet significantly lowered the gene expression of HMG-CoA reductase, which is the rate-limiting enzyme in cholesterol synthesis. Meanwhile, gene expression of SREBP2, a transcription factor of HMG-CoA reductase, was not affected by intake of the SC diet. The SC diets had no significant effects on fecal dry weight or fecal excretions of neutral steroids and fatty acids.

4 Discussion

In the present study, we assessed the effect of dietary SC on body fat mass in rats and then investigated the mechanism of this anti-obesity activity. Our results indicate that increases in body fat mass and hepatic lipid content were suppressed by the SC diet compared with the control diet, clearly implying its anti-obesity activity. This anti-obesity activity of dietary SC can be attributed, at least in part, to an enhancement in carbohydrate oxidation and suppression of lipogenesis in the liver, but not an increase in fecal lipid excretion, under conditions where the rats were fed a similar number of calories.

Three potential mechanisms are speculated to underlie the anti-obesity activity of SC: 1) inhibition of dietary lipid absorption in the small intestine, 2) enhancement of energy expenditure, and 3) suppression of lipogenesis in the body. In terms of inhibition of dietary lipid absorption, several reports have shown that certain mushrooms, such as *Pleurotus eryngii* and *Hericium erinaceus*, inhibit pancreatic lipase activity, leading to an improvement in diet-induced hypertriglyceridemia and reduction in body fat mass, respectively. Therefore, we measured fecal excretion of fatty acids and neutral steroids in this study. However, a similar phenotype was not observed for SC, as no significant changes were observed in the absorption of fatty acids and steroids (Table 4). To address the possibility of enhanced energy expenditure, we conducted respiratory gas analysis after feeding the rats an SC diet for 6 weeks and found that, compared with the control diet, the SC diet promoted carbohydrate oxidation and slightly enhanced energy expenditure in rats (Fig. 1A and 1C). Short-chain fatty acids (SCFAs) are produced from colonic fermentation of dietary fiber by gut microbiota. The association between microbial activity in the gastrointestinal tract, host energy homeostasis, and obesity pathogenesis is becoming increasingly recognized. A series of orphan G protein-coupled receptors (GPRs) bind free fatty acids. Of these, free fatty acid receptor 3 (FFA3/GPR41) is expressed in the intestine and sympathetic nervous system, and is activated by SCFAs. Kimura et al. demonstrated SCFAs directly enhance energy expenditure by increasing sympathetic outflow in mice. In the present study, compared with the control diet, the SC diet significantly higher cecum weight, indicating the fermentability of SC (Table 3). Several studies have found increased cecum SCFA production along with increased cecum weight. Although we did not directly analyze SCFA levels in this study, the increase in cecum weight due to dietary SC may reflect an enhancement of SCFA production in the cecum. Further studies...
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Table 4  Effects of long-term feeding of SC on hepatic lipid metabolism-related enzymatic activity and gene expression levels and fecal lipid excretion in rats (Experiment 2).

|                        | Control       | SC 2%         | SC 4%         |
|------------------------|---------------|---------------|---------------|
| Malic enzyme           | 16.5 ± 2.9    | 9.2 ± 1.3*    | 7.0 ± 1.5†    |
| G6PDH                  | 34.1 ± 7.4    | 12.6 ± 1.6*   | 13.0 ± 4.6†   |
| PAP                    | 21.4 ± 2.2    | 21.3 ± 2.4    | 16.0 ± 1.4    |
| FAS                    | 13.1 ± 1.7    | 10.0 ± 1.5    | 7.3 ± 2.0     |
| Liver mRNA expression  |               |               |               |
| HMG-CoA reductase      | 1.01 ± 0.07   | 0.93 ± 0.09   | 0.74 ± 0.05†  |
| SREBP2                 | 1.01 ± 0.06   | 1.03 ± 0.08   | 0.97 ± 0.07   |
| Fecal dry weight (g/day)| 2.25 ± 0.12  | 2.31 ± 0.11   | 2.75 ± 0.10   |
| Fecal lipid excretion  |               |               |               |
| Neutral steroids (mg/day)| 28.4 ± 2.3  | 24.6 ± 2.4    | 26.4 ± 1.0    |
| Fatty acids (mg/day)   | 240 ± 15      | 226 ± 15      | 271 ± 10      |

Values are presented as mean ± SEM (n = 7-8/group).
*, Significantly different from control (p < 0.05)
†, Significantly different from control (p < 0.05)
G6PDH, glucose-6-phosphate dehydrogenase; PAP, phosphatidate phosphohydrolase; FAS, fatty acid synthase.

are needed to clarify how fermentation of SC in the gut relates to energy metabolism via GPR41.

In addition, dietary SC improved diet-induced hyperinsulinemia in this study (Table 3), suggesting an improvement in insulin sensitivity in the body and suppression of lipogenesis in the liver. A previous report using diabetic KK-Ay mice showed that dietary SC decreases fasting blood glucose and plasma C-peptide levels. GPR41-knockout mice have a lower energy expenditure and glucose tolerance than wild-type mice. Gao et al. reported that dietary supplementation with sodium butyrate improves insulin sensitivity and enhances energy expenditure in mice. Taken together, these suggest that the improvement in insulin sensitivity and enhancement in energy expenditure by dietary SC may be due to activation of GPR41, perhaps through SCFA production.

The SC diet reduced hepatic cholesterol levels compared with the control diet (Tables 2 and 3) without changing the amount of fecal neutral steroid excretion (Table 4). Moreover, hepatic gene expression of HMG-CoA reductase, a rate-limiting enzyme in cholesterol biosynthesis, was decreased in rats, which were fed the SC diet (Table 4). Mushrooms are considered a good source of dietary fiber. In fact, the SC used in this study contained 23.2 g β-glucan per 100 g dry powder. Sina et al. reviewed the effects of β-glucans on cholesterol balance in the body and found β-glucans bind to bile acids, thereby promoting lipid excretion into feces, and induce cholesterol biosynthesis. Given that bile acids have an important role in cholesterol metabolism, further studies are needed to investigate how dietary SC affects lipid metabolism via bile acid metabolism, including synthesis and secretion into bile.

It has been reported that SC contains various bioactive compounds, such as methyl orsellinate, xanthoangelol, 4-hydroxyderricin, sparoside A, hanabiratakelse A, adenosine, and 5α,6α-epoxy-(22E,24R)-ergosta-8(14),22-diene-3β,7β-diol E22. Gao et al. reviewed the effects of SC on hepatic lipid metabolism-related enzymatic activity and gene expression levels and fecal lipid excretion in rats (Experiment 2). Further, Li et al. showed that methyl orsellinate inhibits protein tyrosine phosphatase 1B (PTP1B), a potential drug target in the treatment of diabetes and obesity. Ogawa et al. demonstrated that xanthoangelol reduces hepatic TAG and cholesterol contents in stroke-prone spontaneously hypertensive rats. Zhang et al. reported xanthoangelol and 4-hydroxyderricin inhibit 3T3-L1 adipocyte differentiation via the AMPK and MAPK pathways. Furthermore, Li et al. showed that xanthoangelol reduces abdominal WAT and liver weights and enhances insulin sensitivity in diet-induced obese mice. Bang et al. reported that sparoside A, hanabiratakelse A, adenosine, and 5α,6α-epoxy-(22E,24R)-ergosta-8(14),22-diene-3β,7β-diol E22 potently inhibit mRNA expression of propionate convertase subtilisin/kexin type 9 (PCSK9), a circulating protein that promotes degradation of low-density lipoprotein receptor. As described above, SC contains bioactive compounds that aid in improving obesity, insulin sensitivity, and dyslipidemia. Therefore, we speculate that the anti-
Table 5  Bioactive compounds in SC.

| Compound name                | Functions against obesity, diabetes, and dyslipidemia                                                                 | Experimental design | Reference |
|-----------------------------|----------------------------------------------------------------------------------------------------------------------|---------------------|-----------|
| Methyl orsellinate          | Inhibits PTP1B activity, a target for treatment of obesity and type 2 diabetes                                           | (In vitro assay)    | (38)      |
| Xanthoangelol               | Reduces hepatic cholesterol content by increasing fecal cholesterol excretion                                          | (In vivo)           | (39)      |
|                             | Reduces hepatic triacylglycerol content                                                                             | Spontaneously       |           |
|                             | Reduces abdominal white adipose tissue and liver weight                                                              | Diet-induced obese mice | (41)      |
|                             | Enhances glucose transporter 4 (GLUT4)-dependent glucose uptake                                                      | (In vitro)          |           |
|                             | Inhibits 3T3-L1 adipocyte differentiation via AMPK and MAPK pathways                                                  | 3T3-L1 cells        | (42)      |
|                             |                                                                                                                     | Skeletal muscle cells | (43)      |
| 4-Hydroxyderricin           | Increases GLUT4-dependent glucose uptake                                                                           | (In vitro)          |           |
|                             | Inhibits 3T3-L1 adipocyte differentiation via AMPK and MAPK pathways                                                  | 3T3-L1 cells        | (42)      |
|                             |                                                                                                                     | Skeletal muscle cells | (43)      |
|                             |                                                                                                                     | 3T3-L1 cells        | (40)      |
| Sparpside A                 |                                                                                                                     |                     |
| Adenosine                   |                                                                                                                     |                     |
| Hanabirataclide A           | Potently inhibits mRNA expression of PCSK9, a circulating protein that promotes degradation of low-density lipoprotein receptor | (In vitro)          | (37)      |
|                             |                                                                                                                     | HepG2 cells         |           |
| 5α,6α-epoxy-(22E,24R) -ergosta-8(14),22-diene-3β,7β-diol |                                                                                                                     |                     |           |

obesity effect of dietary SC is exerted through the single, additive, or synergistic actions of these bioactive compounds. Unfortunately, the amounts of these bioactive compounds in SC are not yet known. It would be of interest to examine in the future how these bioactive compounds in SC act in an anti-metabolic syndrome manner.
Effects of Sparassis crispa on Weight of Body Fat in Rats

In conclusion, our results indicate that, in addition to its previously reported anti-diabetic and anti-hypertensive activity, dietary SC has anti-obesity and anti-hyperlipidemic properties and, thus, protects against metabolic syndrome.

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Author Contributions
A.T., Y.N., and B.S. wrote the manuscript. A.T., Y.N., C.N., H.A., and T.H. participated in the experimental work and collected data. A.T. and B.S. analyzed the data. M.S. commented on the manuscript. Y.N. and K.T. contributed to the study design, supervised the study, and commented on the manuscript. All authors have read and approved the final version of the manuscript.

Conflict of Interest
The authors declare that they have no competing interests.

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