Improvement of Diet-induced Obesity by Ingestion of Mushroom Chitosan Prepared from *Flammulina velutipes*

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Abstract: The anti-obesity effects of mushroom chitosan prepared from *Flammulina velutipes* were investigated using an animal model with diet-induced obesity. In this study, 5-week-old imprinting control region (ICR) mice were divided into six groups of 10 mice each and fed different diets based on the MF powdered diet (standard diet) for 6 weeks: standard diet control group, high-fat diet control group (induced dietary obesity) consisting of the standard diet and 20% lard, and mushroom chitosan groups consisting of the high-fat diet with mushroom chitosan added at 100, 500, 1,000, and 2,000 mg/kg body weight. On the final day of the experiment, mean body weight was 39.1 g in the high-fat control group and 36.3 g in the 2,000 mg/kg mushroom chitosan group, compared to 35.8 g in the standard diet control group. In the mushroom chitosan groups, a dose-dependent suppression of weight gain and marked improvements in serum triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol were found. The mushroom chitosan groups showed fewer and smaller fat deposits in liver cells than the high-fat diet control group, and liver weight was significantly reduced. Glutamic oxaloacetic transaminase (GOT) and glutamate pyruvic transaminase (GPT), which are indices of the hepatic function, all showed dose-dependent improvement with mushroom chitosan administration. These results suggested that mushroom chitosan acts to suppress enlargement of the liver from fat deposition resulting from a high-fat diet and to restore hepatic function. The lipid content of feces showed a marked increase correlated with the mushroom chitosan dose. These findings suggest the potential use of mushroom chitosan as a functional food ingredient that contributes to the prevention or improvement of dietary obesity by inhibiting digestion and absorption of fats in the digestive tract and simultaneously promotes lipolysis in adipocytes.

Key words: CrI:j: CD1 (ICR) mice, *Flammulina velutipes* (Curt.:Fr.) Sing. extract, hyperlipemia, mushroom chitosan

1 INTRODUCTION

National expenditures on health care have risen in Japan in recent years as the proportion of elderly people in the population increases. There is a great need for individuals to make efforts to extend their healthy life expectancy—the time they can live healthy, independent lives—through the prevention of lifestyle-related diseases brought on by changes in diet and other lifestyle habits and through maintenance and improvement of mental and physical functions. Against this backdrop, there are increasing expectations for food components with biological regulatory functions related to the physical condition and disease protection, in addition to nutritional and taste functions.

Mushrooms are recognized as having outstanding nutritional function and are also low in calories and high in dietary fiber. There have been several reports that some mushrooms can aid health maintenance and improvement and also prevent lifestyle-related diseases through regulatory functions such as cholesterol reduction\(^1,2\), blood pressure reduction\(^3,4\), and inhibition of high blood sugar level\(^5\).

The *Flammulina velutipes* (Curt.: Fr.) Sing. has been eaten since ancient times and is now the most widely culti-
vated mushroom in Japan. Mushroom chitosan prepared using *Flammulina velutipes* as the raw material has been reported to exhibit a wide range of functions in the body. Unlike chitosan derived from crustaceans, mushroom chitosan contains β-glucan and fatty acid complexes. Trials with human subjects have already shown that intake of mushroom chitosan controls total serum cholesterol and neutral fats5–9, and decreases visceral fat10. In addition, the present authors have reported from *in vitro* trials that mushroom chitosan has lipase inhibitory action and from experiments with Zucker fatty rats that it also has anti-obesity effects11. Furthermore, studies using Tsumura Suzuki obese diabetic (TSOD) mice and cultured cells showed that fatty acid complexes present in mushroom chitosan stimulate the affinity of adipocyte β-adrenergic receptors, thus contributing to the breakdown of visceral fat12,13. The safety of mushroom chitosan has been confirmed through 90-day repeated toxicity tests in mice14 and by a study of excessive consumption in tablet form in humans15, and it is expected to be used as an ingredient in functional foods.

The present study used an animal model with diet-induced obesity to investigate the effects of mushroom chitosan in improving dietary obesity in order to further clarify the effects of mushroom chitosan as a functional food ingredient.

2 EXPERIMENTAL PROCEDURES

2.1 Sample preparation

Chitosan derived from *Flammulina velutipes* used in this study was manufactured by Ricom Corporation, Tokyo, Japan. As in our preclinical and clinical studies conducted to date15, the raw material for this mushroom chitosan was the fruiting bodies of *Flammulina velutipes* grown in Nagano Prefecture and in Zhejiang Province, China. Extraction was carried out at 90°C for 1 h, producing an extract in hot water and the residual fruiting bodies. The residual fruiting bodies (which are rich in chitin, a structural component of the cell wall) were deacetylated by immersion in 50% NaOH (90°C, 5 h), neutralized with lactic acid, and washed with water. They were then ground and treated with glycosyltransferase. Dextrin was added to the enzyme-treated paste and to the hot water extract, and the mixture was homogenized. The homogenate was spray-dried to produce mushroom chitosan.

2.2 Experimental animals

For experiments, 4-week-old male Crlj:CD1 (ICR) mice were purchased from Charles River Laboratories Japan, Inc., Yokohama-shi, and acclimated to the laboratory conditions for 1 week before the start of the experiment. The mice were maintained in groups of 10 in plastic cages (W282 × D451 × H157 mm) in a room with a light (07:00–19:00) and dark (19:00–07:00) cycle, controlled temperature at 20 ± 2°C, and humidity at 60.0 ± 5.0%. During the one week acclimation period, mice had free access to commercial feed (MF powdered diet, Oriental Yeast Co., Ltd.) and tap water passed through a multilayer hollow fiber filter (STC.V2, Toray Industries, Ltd.). Immediately prior to the start of the experiment, each mouse was weighed and allocated to one of the six groups (n = 10) in a manner that minimizes weight variation among the groups.

2.3 Continual administration experiment

This experiment was carried out for six weeks (42 d) on mice between the ages of 5 and 11 weeks. The standard diet was commercially available feed (MF powdered diet, Oriental Yeast Co., Ltd.), during which filtered drinking water and solid food were available with free access. The drinking water was tap water passed through a multilayer hollow fiber filter, and the mice had free access to both food and water.

The mice were divided into six groups: (1) standard diet control group, (2) high-fat diet control group, and (3–6) mushroom chitosan groups which were fed the high-fat diet with varying amounts of mushroom chitosan. Mushroom chitosan was suspended in water for injection (Ohtsuka Chemical Industrial Co., Ltd.) and administered once daily by forced oral administration with control groups receiving only water for injection. The mushroom chitosan dose was set at 100, 500, 1,000, and 2,000 mg mushroom chitosan per 1 kg body weight per day in water for injection. The dose was set in reference to the OECD Guideline for the Testing of Chemicals to represent 10 to 200 times the amount of mushroom chitosan normally ingested by humans.

The present study was carried out in accordance with Takasaki University of Health and Welfare Regulations on Performing Animal Experiments, the Act on Welfare and Management of Animals (Act No. 105), and the Standards Relating to the Care and Management of Laboratory Animals (Ministry of the Environment, Bulletin 88).

2.4 Body weight and amount of food intake

During the course of the experiment, the general condition of the mice was observed daily, and body weight and amount of food intake was measured for each group every 3 d.

2.5 Hematology and blood biochemistry tests

On the final day of the experiment, food was withheld for 24 h, all mice were put in a deeply anesthetized condition with pentobarbital-Na 50 mg/kg, i.p. then a surgical incision was made in the abdomen and blood was collected from the heart. Blood with added EDTA-2K was analyzed for red blood cell (RBC) numbers, hemoglobin (Hb) levels,
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Platelet count (PLT), hematocrit (Ht), total white blood cell count (WBC), and non-esterified fatty acid (NEFA), cholinesterase (CHE), alanine aminotransferase (AST), γ-glutamyl transpeptidase (γ-GTP), lactate dehydrogenase (LDH), creatinine phosphokinase (CPK), blood urea nitrogen (BUN), creatinine (CR), uric acid (UA), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphorus (P), magnesium (Mg), and iron (Fe). All blood analyses were commissioned to and carried out by Health Sciences Research Institute, Inc., Yokohama, Japan.

2.6 Autopsy, organ weight, and histopathological examination

After collecting blood under anesthesia, the animals were euthanized by exsanguination, and the organs and tissues of the whole body were examined macroscopically for abnormalities. At the time of autopsy, the liver and both kidneys were removed, weighed, and fixed in 10% formaldehyde:benzene (1:2 v/v) according to standard procedures. Paraffin sections were prepared following standard procedures, and these sections were hematoxylin-eosin (HE) stained and examined microscopically.

2.7 Measurement of lipids in feces

Total lipids in feces were measured for each group on day 21 following the start of administration and on day 42. Feces were collected from the caged group, freeze-dried, and lipids were extracted using a Soxhlet extractor with alcohol:benzene (1:2 v/v) according to standard procedures and lipid levels measured. 

2.8 Statistical analysis

The experimental results obtained are expressed as means ± standard deviation. Body weight and quantity of food intake was measured for the entire caged groups and not on an individual animal basis. Multiple comparisons were carried out using Dunnett’s test. Significance in the two-tailed test was taken as \( p < 0.05 \).

3 RESULTS

No significant differences in daily food intake were found between groups (Fig. 1). Changes in body weight during the experimental period are shown in Fig. 2, and the high-
fat diet control group exhibited greater mean body weight than the standard diet control group from day 18 onward. From week 3, the dose-dependent effect of mushroom chitosan on suppressing weight gain relative to the high-fat diet control group was observed. At the end of the experimental period, the mean body weight of the high-fat control group was 39.1 g, and the mean body weight of the groups administered 100, 500, 1,000, and 2,000 mg chitosan per kg body weight was 38.8, 37.3, 36.5, and 36.3 g, respectively.

The results of hematology and blood biochemistry tests for each group are shown in Table 1. The results for TG, T-cho, LDL-cho, and HDL-cho are shown by group in Figs. 3, 4, 5, and 6, respectively. No abnormalities were found in the hematology tests. In the blood biochemistry tests, values for TL, TG, T-cho, LDL-cho, LP, and PL, which are parameters related to lipid metabolism, showed dose-dependent reductions in the mushroom chitosan groups relative to the high-fat diet control group. Values for TG and T-cho were markedly lower in all groups administered mushroom chitosan compared to those in the high-fat diet control group. In the 500, 1,000, and 2,000 mg/kg groups, LDL-cho decreased significantly and HDL-cho increased significantly. Notably, the 1,000 mg/kg group presented values that were closest to those of the standard diet control group.

Compared to the high-fat diet control group, the mushroom chitosan groups all showed significant, dose-dependent reductions in AST and ALT which are indicators of liver function as well as reductions in LDH levels, which is an indicator of muscle inflammation and internal organ tissue disorder. Particularly for AST and ALT, the values

Table 1: Effect of the mushroom chitosan on blood serum parameters after 6 weeks of experimental diet intake.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cont-Std</th>
<th>Cont-H</th>
<th>100</th>
<th>500</th>
<th>1000</th>
<th>2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10^6/µl)</td>
<td>926.8 ±19.1 **</td>
<td>1006.1 ±24.1</td>
<td>1047.5 ±44.3 *</td>
<td>979.8 ±19.4</td>
<td>968.0 ±39.5 *</td>
<td>923.9 ±31.0 **</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>20.3 ±0.2 **</td>
<td>21.2 ±0.4</td>
<td>20.9 ±0.5</td>
<td>21.5 ±0.3</td>
<td>20.8 ±0.5</td>
<td>21.4 ±1.2</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>43.8 ±2.4</td>
<td>46.5 ±0.5</td>
<td>46.2 ±0.6</td>
<td>46.2 ±0.6</td>
<td>46.6 ±0.5</td>
<td>46.1 ±0.6</td>
</tr>
<tr>
<td>WBC (µ/l)</td>
<td>27.2 ±0.7</td>
<td>27.5 ±0.6</td>
<td>26.6 ±1.1</td>
<td>27.4 ±0.3</td>
<td>28.6 ±0.8 *</td>
<td>27.8 ±1.3</td>
</tr>
<tr>
<td>PLT (10^3/µl)</td>
<td>118.9 ±6.4 **</td>
<td>107.7 ±1.1</td>
<td>109.3 ±1.2</td>
<td>110.0 ±1.9</td>
<td>108.8 ±2.1</td>
<td>110.1 ±5.4</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>6.39 ±0.1</td>
<td>6.41 ±0.1</td>
<td>6.36 ±0.0</td>
<td>6.36 ±0.0</td>
<td>6.35 ±0.0</td>
<td>6.42 ±0.0</td>
</tr>
<tr>
<td>ALB (g/dl)</td>
<td>4.20 ±0.1</td>
<td>4.20 ±0.1</td>
<td>4.15 ±0.1</td>
<td>4.15 ±0.0</td>
<td>4.14 ±0.0</td>
<td>4.13 ±0.0</td>
</tr>
<tr>
<td>TL (mg/dl)</td>
<td>288.8 ±18.8 **</td>
<td>562.2 ±20.2</td>
<td>427.7 ±8.9 **</td>
<td>346.8 ±12.7 **</td>
<td>295.6 ±10.7 **</td>
<td>273.3 ±15.7 **</td>
</tr>
<tr>
<td>LP (mg/dl)</td>
<td>248.4 ±4.1 **</td>
<td>361.2 ±15.4</td>
<td>307.8 ±12.0 **</td>
<td>260.2 ±7.6 **</td>
<td>248.9 ±7.3 **</td>
<td>256.1 ±7.4 **</td>
</tr>
<tr>
<td>NEFA (mEq/l)</td>
<td>0.60 ±0.0 **</td>
<td>0.62 ±0.0</td>
<td>0.63 ±0.0</td>
<td>0.63 ±0.0</td>
<td>0.62 ±0.0</td>
<td>0.62 ±0.0</td>
</tr>
<tr>
<td>PLT (mg/dl)</td>
<td>244.4 ±15.4</td>
<td>264.0 ±13.6</td>
<td>240.9 ±5.1 **</td>
<td>238.2 ±7.3 **</td>
<td>237.2 ±13.4 **</td>
<td>242.7 ±3.8 **</td>
</tr>
<tr>
<td>LDH (IU/l)</td>
<td>1458.1 ±126.7 **</td>
<td>2133.8 ±118.4</td>
<td>1943.0 ±71.4 **</td>
<td>1666.1 ±113.8 **</td>
<td>1602.8 ±40.1 **</td>
<td>1826.3 ±86.2 **</td>
</tr>
<tr>
<td>CHE (IU/l)</td>
<td>4310.1 ±113.6</td>
<td>4304.4 ±166.3</td>
<td>4069.0 ±94.7 **</td>
<td>3928.1 ±9.8 **</td>
<td>4524.7 ±179.5 **</td>
<td>4335.7 ±76.2</td>
</tr>
<tr>
<td>AST (GOT) (IU/l)</td>
<td>446.6 ±31.5 **</td>
<td>542.1 ±22.8</td>
<td>475.8 ±22.2 **</td>
<td>434.1 ±7.6 **</td>
<td>429.3 ±8.7 **</td>
<td>441.4 ±12.4 **</td>
</tr>
<tr>
<td>ALT (GPT) (IU/l)</td>
<td>130.3 ±7.2 **</td>
<td>145.5 ±6.8</td>
<td>122.7 ±3.6 **</td>
<td>115.7 ±7.6 **</td>
<td>98.2 ±6.6 **</td>
<td>138.0 ±7.5</td>
</tr>
<tr>
<td>r-GTP (IU/l)</td>
<td>63.9 ±2.8 *</td>
<td>67.0 ±2.7</td>
<td>68.7 ±1.0</td>
<td>63.3 ±2.4 **</td>
<td>61.9 ±1.5 **</td>
<td>67.4 ±2.4</td>
</tr>
<tr>
<td>CPK (IU/l)</td>
<td>934.1 ±57.1</td>
<td>1085.9 ±105.3</td>
<td>944.0 ±24.8 *</td>
<td>945.2 ±7.6 *</td>
<td>976.8 ±18.8</td>
<td>973.7 ±48.3</td>
</tr>
<tr>
<td>BUM (mg/dl)</td>
<td>15.3 ±0.4 **</td>
<td>17.2 ±0.5</td>
<td>16.9 ±0.1</td>
<td>15.9 ±0.5 **</td>
<td>15.4 ±0.2 **</td>
<td>15.8 ±0.2 **</td>
</tr>
<tr>
<td>CR (mg/dl)</td>
<td>0.67 ±0.0 **</td>
<td>0.62 ±0.0</td>
<td>0.63 ±0.0 *</td>
<td>0.64 ±0.0</td>
<td>0.65 ±0.0 **</td>
<td>0.67 ±0.0 **</td>
</tr>
<tr>
<td>UA (mg/dl)</td>
<td>6.23 ±0.0</td>
<td>6.24 ±0.0</td>
<td>6.26 ±0.0</td>
<td>6.35 ±0.0 **</td>
<td>6.23 ±0.0</td>
<td>6.37 ±0.1 *</td>
</tr>
<tr>
<td>Na (mEq/l)</td>
<td>135.2 ±1.9</td>
<td>136.6 ±2.0</td>
<td>136.3 ±1.8</td>
<td>129.3 ±1.6 **</td>
<td>143.1 ±2.2 **</td>
<td>142.2 ±1.9 **</td>
</tr>
<tr>
<td>K (mEq/l)</td>
<td>6.7 ±0.1</td>
<td>6.8 ±0.1</td>
<td>6.3 ±0.1 **</td>
<td>6.7 ±0.2</td>
<td>6.8 ±0.1</td>
<td>6.5 ±0.2 **</td>
</tr>
<tr>
<td>Cl (mEq/l)</td>
<td>107.7 ±0.8 **</td>
<td>115.8 ±3.8</td>
<td>108.3 ±1.2 **</td>
<td>111.5 ±4.1</td>
<td>109.1 ±3.5 **</td>
<td>109.9 ±2.2 **</td>
</tr>
<tr>
<td>Ca (mEq/l)</td>
<td>9.2 ±0.1 **</td>
<td>9.6 ±0.1</td>
<td>9.2 ±0.1 **</td>
<td>8.7 ±0.1 **</td>
<td>9.3 ±0.2 **</td>
<td>9.3 ±0.1 **</td>
</tr>
<tr>
<td>P (mEq/l)</td>
<td>7.2 ±0.1</td>
<td>7.2 ±0.1</td>
<td>7.3 ±0.2</td>
<td>7.3 ±0.2</td>
<td>7.3 ±0.1</td>
<td>7.3 ±0.1</td>
</tr>
<tr>
<td>Mg (mEq/l)</td>
<td>2.6 ±0.1 **</td>
<td>2.2 ±0.1</td>
<td>2.4 ±0.1 **</td>
<td>2.5 ±0.1 **</td>
<td>2.4 ±0.1 **</td>
<td>2.4 ±0.1 **</td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>146.8 ±0.8 **</td>
<td>152.8 ±1.3</td>
<td>150.8 ±1.3</td>
<td>152.0 ±3.9</td>
<td>145.3 ±2.5 **</td>
<td>153.8 ±3.0</td>
</tr>
</tbody>
</table>

Values are mean ± SD for 10 mice. Cont-Std, standard diet control group; Cont-H, high fat diet control group; 100, high fat diet with mushroom chitosan at 100 mg/(kg·d); 500, high fat diet with mushroom chitosan at 500 mg/(kg·d); 1000, high fat diet with mushroom chitosan at 1000 mg/(kg·d); 2000, high fat diet with mushroom chitosan at 2000 mg/(kg·d).

Significantly different from high fat diet control group: *, p < 0.05; **, p < 0.01.
were elevated in the high-fat diet control group at 542.1 ± 22.8 and 145.5 ± 6.8 IU/l, respectively, and markedly reduced in the 1,000 mg/kg mushroom chitosan group at 429.3 ± 8.7 and 98.2 ± 9.6 IU/l, respectively, which were lower than the standard diet control group (446.6 ± 31.5 and 130.3 ± 7.2 IU/l, respectively). The values for TP and ALB, which are indices for the diagnosis of hepatic or renal dysfunction, showed no changes that would be indicative of disorder. The values for BUN and CR, which are indices of renal dysfunction, were significantly higher in the high-fat diet control group than the standard diet control group, but these values were dose-dependently lower in the 1,000 and 2,000 mg/kg mushroom chitosan groups, approaching values observed for the standard diet control group.

Liver weight is depicted in Fig. 7. The mushroom chitosan groups tended to show suppression of liver weight increase with respect to the high-fat diet group, and significant reductions were observed in the 500, 1,000, and 2,000 mg/kg mushroom chitosan groups. HE stained sections of liver are shown in Fig. 8. Fat deposition in the area surrounding the hepatic artery in the centrilobular zone of the liver tended to be lower in the 1,000 and 2,000 mg/kg administration groups.

The lipid content of feces in mushroom chitosan 100, 500, 1,000, and 2,000 mg/kg administration groups was significantly greater than in the high-fat diet control group. Compared to the lipid content of feces in the high-fat diet control group of 4.37 %, lipid excretion in the feces was highest in the 1,000 mg/kg administration group at 7.42 %.

4 DISCUSSION

In order to investigate the effects of mushroom chitosan...
on dietary obesity, 5-week-old ICR mice were continuously fed a diet with 20% added lard and varying concentrations of mushroom chitosan derived from *Flammulina velutipes* for 6 weeks. Compared to the standard diet group, the ICR mice fed a high-fat diet showed a marked increase in body weight and significant increases in serum TG and blood lipids such as T-cho and LDL-cho. Furthermore, enlargement of the liver due to fat deposition was observed in the high-fat diet group, and there was a marked increase in liver weight, indicating that ingestion of a high-fat diet led to dietary obesity in the high-fat diet control group.

In groups that were administered mushroom chitosan while being fed a high-fat diet, dose-dependent suppression of body weight increase and marked reduction in serum TG and T-cho values were observed. Further, the 500, 1,000, and 2,000 mg/kg mushroom chitosan groups exhibited marked reductions in LDL-cho values and increases in HDL-cho values, and they also showed a decrease in lipid droplets in liver cells and a significant reduction in liver weight. Thus, mushroom chitosan showed dose-dependent anti-obesity effects. No differences in food intake were found among groups in this study, which implies that the anti-obesity effects were a result of the dose-dependent effects of mushroom chitosan.

Dietary fats in the duodenum are broken down by pancreatic lipase into fatty acids and monoglycerides, which are absorbed by small intestinal epithelial cells. In the present experiment, the lipid content of feces was clearly greater in all experimental groups administered with mushroom chitosan than in the high-fat diet control group. This result agrees with that of a previous experiment in which 6-week-old Zucker fatty rats were given repeated oral administration of the same doses of mushroom chitosan for 10 weeks, and that of another study in which 8-week-old Wistar rats had free access to a high-fat diet containing 5% mushroom chitosan for 3 weeks.

Chitosan binds to various substances in the digestive tract causing them to be excreted and is reported to bind to bile acid and to retain fats in the digestive tract. The mushroom chitosan used in the present experiment is reported to inhibit lipase activity *in vitro* and to suppress absorption of fats from the intestine by forming a thin coating on the intestine mucous membrane *in vitro*. From these observations, it may be conjectured that the increase in fat excretion in the feces in the mushroom chitosan administration groups in the present experiment was due to inhibition of lipase, which breaks down dietary fat, as well as suppression of fat absorption in the digestive tract.
stat, which suppresses the breakdown of fats by inhibiting the action of lipase such that fats are excreted from the body, has already been put to practical clinical use as an anti-obesity agent. The present results suggest that the effects of mushroom chitosan to promote dietary fat excretion are useful for the improvement of obesity.

β-glucan, which is a polysaccharide component of the cell wall in microorganisms and plants, has the effect of lowering blood cholesterol by promoting excretion of cholesterol in the feces, inhibiting reabsorption of bile acid, and inhibiting cholesterol synthesis in the liver. In the present experiment, all mushroom chitosan administration groups showed significantly lower T-cho and TG than the high-fat diet control group. In addition, the 500, 1,000, and 2,000 mg/kg mushroom chitosan groups showed significantly lower LDL-cho and markedly higher HDL-cho than the high-fat diet control group. Mushroom chitosan contains β-glucan, which is a dietary fiber, and chitosan, which has the effect of binding to bile acid. It may therefore be conjectured that the reasons for the reduction in cholesterol were the combined actions of β-glucan and chitosan to reduce cholesterol and neutral lipids by binding to bile acid, thus inhibiting micelle formation, as well as the promotion of catabolism of cholesterol into bile acid through inhibition of bile acid reabsorption. Increased blood cholesterol is a cause of arteriosclerosis, and LDL-cho is known to bring about arteriosclerosis by transporting cholesterol to peripheral cells. On the other hand, HDL-cho is involved in the transport of cholesterol from peripheral tissues to the liver, making low values undesirable. In the present study, mushroom chitosan was shown to correct disorders of blood lipid metabolism due to dietary obesity by not only reducing TG, T-cho, and LDL-cho but by also increasing

Fig. 7 Effect of mushroom chitosan on liver weight after 6 weeks of experimental diet intake. Values are mean ± SD for 10 mice. Cont-Std, standard diet control group; Cont-H, high fat diet control group; 100, high fat diet with mushroom chitosan at 100 mg/(kg·d); 500, high fat diet with mushroom chitosan at 500 mg/(kg·d); 1000, high fat diet with mushroom chitosan at 1000 mg/(kg·d); 2000, high fat diet with mushroom chitosan at 2000 mg/(kg·d). Significantly different from high fat diet control group: *, p<0.05; **, p<0.01.

Fig. 8 Effect of mushroom chitosan on hepatic lipid droplets shown by HE staining of liver tissue slices. Pathological Examination (pathological analysis of hepatic lipid droplets) was performed by HE staining. Tissue slices are shown for (a) standard diet control group, (b) high fat diet control group, (c) high fat diet with mushroom chitosan at 100 mg/(kg·d), (d) high fat diet with mushroom chitosan at 500 mg/(kg·d), (e) high fat diet with mushroom chitosan at 1000 mg/(kg·d), (f) high fat diet with mushroom chitosan at 2000 mg/(kg·d).
Fig. 9 Effect of mushroom chitosan on the lipid content in feces after 6 weeks of experimental diet intake. Values are mean ± SD for 10 mice. Cont-Std, standard diet control group; Cont-H, high fat diet control group; 100, high fat diet with mushroom chitosan at 100 mg/(kg·d); 500, high fat diet with mushroom chitosan at 500 mg/(kg·d); 1000, high fat diet with mushroom chitosan at 1000 mg/(kg·d); 2000, high fat diet with mushroom chitosan at 2000 mg/(kg·d). Significantly different from high fat diet control group: *, p < 0.05; **, p < 0.01.

HDL-cho, so that these values approach normal for each parameter. Furthermore, the effects of mushroom chitosan showed dose-dependent activity only in the range of restoring parameters to normal levels. Administration of mushroom chitosan at higher levels was not observed to push parameter values beyond normal levels, demonstrating the safety of administration with regard to the parameters tested here. In a prior study of Wistar and DahlS rats in which high salt-containing chitosan was administered, hypertensive effects of Ca excretion through feces were reported. In this previous study, no changes in blood concentration or hypertensive effects were observed, whereas in the present study, the values for Na and K changed only in the mushroom chitosan groups. The mechanisms of action are not clear, and further investigations of changes in blood biochemistry parameters related to electrolyte balance are needed.

Liver weight significantly decreased in a dose-dependent fashion because of mushroom chitosan administration. In addition, liver pathology images show hepatic enlargement due to fat deposition in liver parenchymal cells of the high-fat diet control group, and reduction of lipid droplets in liver cells was found because of mushroom chitosan administration. The marked reduction in liver weight and the improvement in fat deposition observed in liver tissue in the present study are in accordance with results of a prior study by the authors using Zucker fatty rats. The blood biochemistry tests revealed significant, dose-dependent reductions in the enzyme activity values of GOT, GPT and LDH, which are indices of liver function, suggesting that mushroom chitosan inhibits the accumulation of fat in the liver of mice fed a high-fat diet, and therefore has the effect of improving liver function. The ability of mushroom chitosan to reduce excess body fat has been shown in experiments on humans: in a double-blind trial, reduction of fat mass was found in human subjects with BMI ≥ 25, and large fat reduction effects were reported in healthy female university students with higher than average body fat. In addition, a study in which mushroom chitosan was added to a culture of rat visceral adipocyte precursor cells in order to study its effects on fat accumulation, microscopic observation revealed marked reduction in lipid droplets and significant decreases in TG in the vicinity of the DNA within cells. In a study using TSOD mice, which are a model for metabolic syndrome, administration of mushroom chitosan and fatty acid complex components of mushroom chitosan resulted in significant decreases in the quantity of epididymal adipose tissue. This suggests the involvement of fatty acid complexes in the visceral fat reduction effect of mushroom chitosan. Furthermore, as a mechanism for specific visceral fat reduction effect, mushroom chitosan is reported to have relatively high affinity for β-adrenergic receptors. It may be conjectured that the reduction in size and number of lipid droplets in liver cells that was observed in the present study with mushroom chitosan administration was due to lipolysis by the fatty acid complexes present in the mushroom chitosan.

These findings show that, in response to dietary obesity brought induced by intake of a high-fat diet, mushroom chitosan acts to suppress body weight increase by normalizing metabolism of lipids such as serum TG or T-cho and by suppressing the accumulation of excessive fats in the liver and other organs. Moreover, the results suggest that mushroom chitosan improves dietary obesity not only by breaking down fats but also by suppressing digestion of fats and reabsorption of bile acid in the digestive tract. We determined that mushroom chitosan effects are dose-dependent and are safe at levels beyond those required to maintain normal levels.

5 CONCLUSION

The effects of mushroom chitosan on dietary obesity were investigated by feeding mice a high-fat diet produced by adding 20% lard to normal feed and simultaneously administering mushroom chitosan for 6 weeks. Mushroom chitosan administration groups showed marked suppres-
tion of body weight increase, as well as suppression of serum TG and T-cho increases that typify high-fat diet intake. In addition, obvious LDL-cho decrease and HDL-cho increase were observed, suggesting that mushroom chitosan acts to improve blood lipid metabolism. Enlargement of the liver and notable increase in liver weight due to fat deposition were observed in the high-fat diet control group, and the size and number of lipid droplets in liver cells decreased because of mushroom chitosan administration. There was also an obvious decrease in liver weight. The fat content of feces showed a clear increase because of mushroom chitosan administration. The effects of mushroom chitosan administration, particularly in the 500, 1,000, and 2,000 mg/kg groups, were remarkable and suggested dose-dependent action. The results therefore suggest that mushroom chitosan acts to improve dietary obesity by inhibiting digestion and absorption of fats and reabsorption of bile acid in the digestive tract, and by breaking down fats.

ACKNOWLEDGMENT
This work was supported by Grant-in-Aid for Scientific Research No.26450235.

References