Anti-hyperliposis Effect of Maitake Fruit Body (*Grifola frondosa*). I

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Experimental rat models (5-week-old Sprague-Dawley rats) with hyperlipemia were prepared by feeding high-cholesterol feed containing sodium cholate and casein as a protein source. Dried maitake (*Grifola frondosa*) powder was mixed with the basic high-cholesterol feed and the serum lipids were periodically measured. Values of cholesterol, triglyceride and phospholipid in serum of rats in the maitake-feed group were suppressed by 0.3–0.8 times those in animals fed the basic feed, the latter values being close to those in rats given normal feed. The value of high density lipoprotein (HDL)-cholesterol in serum which is generally reduced by the ingestion of high-cholesterol feed remained the level it was at the beginning of the experiment. Weights of extirpated liver and epididymal fat-pads were significantly less (0.6–0.7 times) than those in the basic feed group, indicating that maitake inhibits lipid accumulation in the body. Liver lipids were also measured and the values were found to be decreased by maitake administration as true of serum lipid, suggesting maitake has an anti-liver lipid activity. Measurement of the amount of total cholesterol and bile acid in feces showed, the ratio of cholesterol-excretion had increased 1.8 times and bile acid-excretion 3 fold by maitake treatment. From these results, it is believed that maitake helps to improve the lipid metabolism as it inhibits both liver lipid and serum lipid which are increased by the ingestion of high-fat feed.

Key words hyperlipemia; maitake; lipid metabolism

Some mushrooms in Basidiomycotina reportedly have the ability to lower serum cholesterol concentration. Kaneda *et al.*¹⁾ and Tokita *et al.*²⁾ found that eritadenine in shiitake (*Lentinus edodes*) lowers the serum cholesterol concentration in rats. It is also reported that hiratake (*Pleurotus ostreatus*) has the same ability^{3–5)} and that mannentake (*Ganoderma lucidum*) can lower blood pressure and serum cholesterol concentration of spontaneously hypertensive rats.⁶⁾ The authors previously discussed preventive effects of maitake against hypertension and diabetes.^{7,8)} During those experiments, it was observed that body weight increase in a maitake-feed group was apparently less than that in a normal-feed group. To determine the reason for the slight body weight increase by maitake, we prepared experimental animal models with high serum lipids and examined the activity of maitake on the lipid metabolism.

MATERIALS AND METHODS

Animal Models Four-week-old male Sprague-Dawley (SD) rats were purchased from Clea Japan Inc. and raised for 7 d on normal feed. Normally grown rats weighing around 140 g were selected at random and divided into groups so that average body weight was equalized.

Raising Conditions Isolated rats were housed in steel-mesh cages and raised in a specific-pathogen-free (SPF) animal laboratory with room temperature of $24 \pm 1^\circ\text{C}$, humidity 55% and day/night cycle of 12 h (daytime; 7:00–19:00). Animals were fed *ad libitum*.

Composition of Diets Hypercholesterolemic diets containing 1% cholesterol and 0.3% sodium cholate using casein as protein source were used (Table 1). Mineral and vitamins were balanced according to a normal commercial diet (CE-2, Clea Japan Inc.). Dried maitake powder (i.d. 200 μm) was mixed in an amount 20% (w/w) of total diet weight, and adjusted with α -cornstarch. Solid pellet

diets were made according to the commercial diet of CE-2 and there was no difference in the pellet shape.

Animal Treatment Blood was collected periodically by cardiopuncture from rats lightly anesthetized with diethyl ether and centrifuged for 10 min at 3000 rpm to separate the serum. Prior to the final blood take from the heart, the rats were not fed for 12 h; they were then sacrificed under anesthesia with sodium barbital. Immediately thereafter, livers and epididymal fat-pads were extirpated, washed with saline, weighed, and liver was preserved in a refrigerator at -80°C .

Serum Lipids Using a commercial kit of total cholesterol (Cholesterol E-Test), free cholesterol (Free Cholesterol E-Test), HDL-cholesterol (HDL-Cholesterol Test), phospholipids (Phospholipid B-Test) and triglycerides (Triglyceride E-Test) (Wako Pure Chemical Industries, Ltd., Japan) were measured. Liver lipids were measured in the same way as the serum after extracting all lipid using the method of Folch *et al.*⁹⁾

Feces The feces were gathered on the 7th, 14th and 30th day after administration of feed and the wet-weight determined. After complete drying by freezing *in vacuo*,

Table 1. Composition of Hypercholesterolemic Diet

Component	Maitake (%)	Control (%)
Casein	20.0	20.0
Sucrose	20.0	20.0
Cornstarch	15.7	35.7
Lard	10.0	10.0
Corn oil	2.0	2.0
Cellulose	3.0	3.0
Sodium cholate	0.3	0.3
Cholesterol	1.0	1.0
Maitake	20.0	0.0
Vitamin mixture	1.0	1.0
Mineral mixture	7.0	7.0

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the dried-weight and lipid content were measured.

Excretion Rate of Cholesterol The excretion rate was determined by measuring total cholesterol content ingested in food and excreted in feces, the excretion rate (%) of cholesterol which was transferred from food to excrement was calculated.

Statistical Examination Results of the experiments are indicated by average values $\pm$ standard allowances and Student's *t*-test was employed to examine the significant difference between the measured values of each group.

RESULTS

The effects of maitake on lipid metabolism were examined. Figure 1 shows the change in body weight when 5-week-old male SD rats were fed hypercholesterolemic diets (to make hyperlipemia model rats) and the same diet containing 20% maitake powder for 25 d, respectively. Rapid weight increase was seen in the control group fed the hypercholesterolemic diet, while weight was suppressed in the maitake-fed group, indicating that the weight increase normally caused by hypercholesterolemic diet was inhibited by maitake. Diet amounts during the period were 21.2 ± 1.6 g/d for the control group and 22.8 ± 1.2 g/d for the maitake fed group.

Weight gain after 25 d was 179.0 ± 10.2 g for control group and 130.6 ± 11.4 g for maitake group. Food intake during that period was 499.4 ± 22.3 g and 494.2 ± 17.3 g. The food efficiencies in terms of body weight increase per 1 g of food intake were 0.357 ± 0.03 and 0.264 ± 0.01 , indicating that maitake inhibits the body weight increase experienced when high lipid foods are ingested.

Livers and epididymal fat-pads were extirpated for examination from rats raised for 11 d on hypercholesterolemic diet (control group) and those on hypercholesterolemic diet containing maitake (Table 2). Liver weight was less by 0.68 times than that of control and epididymal fat-pads were less by 0.63 times than that of control, respectively. Weight ratio of these organs to total body weight were also low, with significant difference, as little as 0.88 times and 0.79 times. The livers in control group

Fig. 1. Effect of Maitake Intake on Body Weight

Significant difference (Student's *t*-test): ** $p < 0.01$. ●, Maitake; ○, control (6 rats in a group).

were considerably swollen, color and luster were turned to be those of fatty liver. These features were not observed in the maitake-fed group.

Cholesterolemic diets are known to raise the accumulation of liver lipids. Therefore, the lipids contained in the homogenized liver were examined. As shown in Table 3, the values of triglyceride, total cholesterol and free cholesterol were reduced by feeding maitake with significant difference by 0.46 times, 0.54 times and 0.65 times, respectively. However, there was almost no difference in HDL-cholesterol and phospholipid.

Figures 2 and 3 indicate the results of serum lipid changes on 5-week-old male SD rats after feeding hypercholesterolemic diet as the control and the diet with maitake for 21 d. Triglyceride value in the maitake group was 0.73 times that in the control group on the 14th day after administration and phospholipid was 0.86 times (Fig. 2). On the 21st day, triglyceride value was 0.82 times and phospholipid value was 0.76 times, indicating a decrease in the maitake group with significant difference. Figure 3 shows the variation in total-cholesterol and free-cholesterol values; rapid increases in both were observed after 2–3 d with the hypercholesterolemic diet. The maitake-fed group, however, demonstrated lower values than the control group: 0.81, 0.28 times in total-cholesterol and 0.55 and 0.36 times in free-cholesterol on the 7th and 21st day, respectively. The difference of the two groups was constant with the maitake group always showing lower values.

Figure 4 shows the variation in HDL-cholesterol changes. Values in the maitake group maintained the same level for two weeks after the experiment started. On the contrary, values in the control group were 0.66 times on the 14th day and 0.69 times on the 21st day, a decrease with significant difference.

Figure 5 shows the amount of total cholesterol content contained in serum which was calculated as follows¹⁰⁾:

$$(\text{body weight} \times 0.074 / \text{specific gravity of blood}) \times 0.6a$$

Table 2. Effect of Maitake on Liver Weight and Epididymal Fat-pads

Parameter	Control	Maitake
Liver weight (g)	13.01 ± 0.55 (1.00)	8.86 ± 0.51 (0.68)**
Liver weight/body weight (%)	4.91 ± 0.40 (1.00)	4.32 ± 0.19 (0.88)**
Fat-pads (g)	2.72 ± 0.53 (1.00)	1.71 ± 0.17 (0.63)*
Fat-pads/body weight (%)	1.11 ± 0.16 (1.00)	0.88 ± 0.06 (0.79)*

Significant difference (Student's *t*-test): * $p < 0.05$, ** $p < 0.01$. Each value indicates the average of 6 rats.

Table 3. Effect of Maitake on Lipid Contents in Liver

Parameter	Control	Maitake
Triglyceride (mg/g liver)	41.1 ± 2.3 (1.00)	19.0 ± 0.8 (0.46)**
Total-cholesterol (mg/g liver)	22.8 ± 0.5 (1.00)	12.3 ± 0.6 (0.54)**
Free-cholesterol (mg/g liver)	6.0 ± 1.0 (1.00)	3.9 ± 0.7 (0.65)**
HDL-cholesterol (mg/g liver)	0.77 ± 0.1 (1.00)	0.80 ± 0.1 (1.03)
Phospholipid (mg/g liver)	7.4 ± 1.1 (1.00)	7.5 ± 1.2 (1.01)

Significant difference (Student's *t*-test): ** $p < 0.01$. Each value indicates the average of 6 rats.

Fig. 2. Effect of Maitake-Treatment on Triglyceride and Phospholipid in Serum

Significant difference (Student's *t*-test): * $p < 0.05$, ** $p < 0.01$. ●, Maitake; ○, control (6 rats in a group).

Fig. 3. Effect of Maitake-Treatment on Total-Cholesterol and Free-Cholesterol in Serum

Significant difference (Student's *t*-test): * $p < 0.05$, ** $p < 0.01$. ●, Maitake; ○, control (6 rats in a group).

Fig. 4. Effect of Maitake on the Contents of HDL-Cholesterol in Serum

Significant difference (*t*-test): * $p < 0.05$, ** $p < 0.01$. ●, Maitake; ○, control (6 rats in a group).

Fig. 5. Contents of Total-Cholesterol in Whole Plasma of Rat after Treatment with Maitake

Significant difference (*t*-test): ** $p < 0.01$, *** $p < 0.001$ (6 rats in a group).

a is cholesterol content (mg) in 1 ml serum, 0.6 was used on the assumption that serum is 60% of total blood, specific gravity of blood is set as 1.027 and 0.074 is the blood ratio to body weight. As shown in the figure, content in the maitake group was 0.53 times on the 14th day, 0.46 times on the 21st and 0.27 times on the 28th day.

Next, we examined the amount of total-cholesterol and bile-acid in feces (Figs. 6 and 7). The ratio of cholesterol-excretion was increased by 1.8 times on the 14th day by maitake treatment and bile acid-excretion was 3 times higher on the 28th day.

DISCUSSION

The mushroom fruit bodies of *Basidio mycotonia* in fungi were reported to have the ability to lower cholesterol in blood serum. The authors have reported that maitake mushroom (*Grifola frondosa*) has various physiologic activities.¹¹⁾ Especially, the inhibition of body weight increase was observed by maitake in animal models during the studies of its anti-hypertensive and anti-diabetic effects.^{7,8)} It was believed that the body weight increase

Fig. 6. Effect of Maitake-Treatment on Total-Cholesterol Values in Feces

Significant difference (*t*-test): ****p* < 0.001 (6 rats in a group).

Fig. 7. Effect of Maitake-Treatment on Total-Bile-Acid Values in Feces

Significant difference (*t*-test): ****p* < 0.001 (6 rats in a group).

was suppressed by the improvement of lipid-metabolism. Some foods containing β -glucan which are rich in water-soluble dietary fibers were also reported to have ability to lower serum cholesterol concentration of rats.¹²⁾ Therefore, the experiment was conducted assuming that maitake which contains a good deal of β -glucan¹¹⁾ might influence the lipid metabolism.

First, hypercholesterolemic diet was prepared as the basic diet to make animal models with hyperlipemia. The model with hyperlipemia for the experiment was confirmed to have a considerably higher amount of lipids such as total serum cholesterol and triglyceride, and to accumulate lipid. When maitake was added to the basic cholesterol-olemic diet, the increase of the serum lipid, triglyceride, phospholipid and cholesterol was inhibited (Figs. 2 and 3). This led us to assume that the increase in liver weight and liver lipid accumulation were also inhibited.

Next, the HDL-cholesterol contents were examined (Fig. 4). Generally, lower HDL-cholesterol values were indicated when hypercholesterolemic diets were administered in this experiment. HDL-cholesterol has the function of removing LDL (low density lipoprotein)-cholesterol from tissues and sending it to liver thus preventing

cholesterol accumulation on the arterial walls. Thus, low HDL-cholesterol may lead to arteriosclerosis and myocardial infarction. Some mushrooms like shiitake (*Lentinus edodes*) maintain a high level of HDL-cholesterol even in the hypercholesterolemic diets and others like yamabushitake (*Hericum erimaceum*) lower HDL-cholesterol values. Maitake inhibits the lowering HDL-cholesterol.

Increase of cholesterol amounts is known to be the cause of atherosclerosis and ischemic heart diseases, but maitake significantly reduced the amounts. It was also observed that the amount of total serum cholesterol was reduced to less than 1/2 that in the control group; this result is very important. Triglyceride, a "neutral lipid," is affected by diet very easily, and may cause obesity and other diseases as does a high amount of cholesterol. It is possible that, in the case of high triglyceride level in the blood, there is a dangerous factor leading to diabetes with symptoms of abnormal glucose tolerance and insulin resistance, both of which are related to visceral fat obesity. Another dangerous factor is cardiovascular disease including the development of thrombosis. However, maitake maintained the triglyceride in blood lower than those in the control group, and the value of triglyceride content in the liver was about half of that in the control group.

As mentioned, it was observed that maitake affects the lipid-metabolism and inhibits the increase of harmful lipids in serum and liver. It was also significant that, as seen in Table 2, epididymal fat-pads were decreased by maitake and the occurrence of fatty liver was also inhibited.

Furthermore, as shown in Fig. 6, excreted cholesterol values in feces of the maitake group was elevated on the 14th day but decreased to below that level on the 30th day. Bile acid values in the maitake group were lower on the 7th and 14th day but rapid increase was observed on the 30th day. These results suggested that maitake accelerated the excretion of cholesterol in the early stage of administration and also raised the catabolism of cholesterol to bile acid and its excretion in feces. There is no relationship, however, between the excretion values of cholesterol or bile-acid in feces and the content of fat in blood, liver or body.

Maitake's anti-hyperlipemia activities thus were due to the acceleration of cholesterol and bile acid excretion, and our tests also showed that it is involved in accelerating cholesterol conversion into bile-acid. We presume that maitake is concerned in lipid metabolism through other mechanisms as well. This will be further explored to clarify not only the suppression of cholesterol-synthesis in liver, but also the inhibition of cholesterol-absorption in digestive organs and liver.

From all results, we believe that maitake accelerates the function of lipid-metabolism in the body.

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