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Effect of a *Pholiota adiposa* Extract on Fat Mass in Hyperlipidemic Mice

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The purpose of this study was to investigate the effect of a *Pholiota adiposa* extract on fat mass in hyperlipidemic mice fed on a high-fat diet. The water extracts from *P. adiposa* (ASI 24018) were not affected in the total triglyceride contents and epididymal fat mass in mice fed on a high-fat diet, but the retroperitoneal fat mass decreased significantly. This result suggests that the *P. adiposa* extract may be a potential candidate for use as a functional food that can act as a prophylactic against hyperlipidemia. However, the *P. adiposa* extract showed no effect in the total triglyceride contents and epididymal fat mass.

KEYWORDS: Antihyperlipid, Hepatic lipid, Hyperlipidemic mice, *Pholiota adiposa* (ASI 24018), Serum

Mushrooms have become more and more attractive as gourmet and functional foods, as well as a source for the development of new drugs. They provide potentially beneficial effects for several of the most common human diseases, such as cancer (Koo *et al.*, 2006). *Pholiota adiposa KUMM,* commonly called "yellow cap fungus", is classified as *Pholiota* genus of the *Strophariaceae* family and is found exclusively in Korea, Japan, China, Europe, and North America. Few studies have focused on the pharmaceutical effect of *Pholiota adiposa*, except with respect to its antibiotic or antitumor activity.

Hyperlipidemia refers to a high level of lipids (fats or cholesterol) circulating in the blood. An increase in the blood levels of triglycerides, as well as an increase in cholesterol, can lead to pancreatic and cardiovascular diseases, such as arteriosclerosis, hypertension, obesity, diabetes, and functional depression of some organs (Kim *et al.*, 2005). The correlation of plasma cholesterol levels with coronary heart disease was confirmed by early observations that cholesterol is the major component of atherosclerotic plaque (Kim *et al.*, 2005; Hargis, 1988; Bischoff and Rodwell, 1992).

One of the rate-limiting enzyme in the biosynthetic pathway of cholesterol is HMG-CoA reductase (β-hydroxy-β-methylglutaryl Coenzyme A reductase, E.C 1.1.1.34). This enzyme catalyzes the four-electron reduction of HMG-CoA into CoA and mevalonate, along with the oxidation of two molecules of NADPH (Daniel *et al.*, 1999a, b; Frimpong *et al.*, 1993, 1994a, b).The action of HMG-CoA reductase reduces HMG-CoA to mevalonic acid by using NADPH as the hydrogen donor. The end-product molecule, cholesterol, acts as a feedback inhibitor of HMG-CoA reductase to regulate the intracellular and intercellular levels of cholesterol.

Yu *et al.* (2006) investigated the screening of a potent antihyperlipidemic HMG-CoA reductase inhibitor-producing *P. adiposa* (ASI 24018) and the extraction condition of a HMG-CoA reductase inhibitor from its fruiting body. We now investigate how *P. adiposa* (ASI 24018) extracts affect the fat mass in hyperlipidemic mice on a high-fat diet.

Materials and Methods

We used the potent antihyperlipidemic compound-containing mushroom *P. adiposa* (ASI 24018), which was selected in a previous paper (Yu *et al.*, 2006).

Male ICR mice (3 weeks old) were purchased from DaeHan Biolink Co., Ltd., (Chungbuk, Korea) and housed individually in suspended wire-mesh stainless steel cages in a temperature-controlled animal room. After one week of adaptation, the mice were assigned to 5 groups: NF, normal-fat diet (n = 8); HF, high-fat diet (n = 8); HF1, high-fat diet supplemented with 0.1% of a *P. adiposa* extract (n = 8); HF2, high-fat diet supplemented with 0.5% of a *P. adiposa* extract (n = 8); HF3, high-fat diet supplemented with 1.0% of a *P. adiposa* extract (n = 8). Table 1 shows the contents of experimental diets.

Analytical procedure. At 7 weeks, the mice were fasted overnight and their blood was drawn from their eye vein. The serum was left standing for 30 minutes at room temperature and then centrifuged (3,000 rpm, 20 min). The mice were killed. We then bled and weighed the mice and collected the liver, kidney and epididymal fat pad of each mouse. The serum and hepatic tissues were stored at −70°C until they were measured for lipid content such as

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Table 1. Ingredient composition of several *Pholiota adiposa* diets used in this study

Ingredient (g/kg diet)	NF*	HF	HF1	HF2	HF3
Corn starch	529.5	389.5	388.5	384.5	379.5
Casein	200	200	200	200	200
Sucrose	100	100	100	100	100
Soybean oil	70	70	70	70	70
Lard		130	130	130	130
Cholesterol		10	10	10	10
α-Cellulose	50	50	50	50	50
Mineral mix (AIN-93M-MX)	35	35	35	35	35
Vitamin mix (AIN-93-VX)	10	10	10	10	10
DL-methionine	3	3	3	3	3
Choline bitartrate	2.5	2.5	2.5	2.5	2.5
Pholiota adiposa			1	5	10

*NF, normal fat diet; HF, high fat diet; HF1, high fat diet supplemented with 0.1% *P. adiposa* extract; HF2, high fat diet supplemented with 0.5% *P. adiposa* extract; HF3, high fat diet supplemented with 1.0% *P. adiposa* extract.

the total triglyceride and fat (Oh *et al.*, 2004; Jeon *et al.*, 2004).

The serum triacylglycerol and total cholesterol were determined with the aid of a Dry Biochemical Analyzer (Ektachem, USA). We then used established methods (Lee *et al.*, 2003) to measure the hepatic total lipid, the triacylglycerol, and the total cholesterol. The total lipid in the liver which was extracted by using the method of Folch *et al.* (1957), was gravimetrically determined. Finally, we used the method described by Biggs *et al.* (1975) to measure the liver triacylglycerol and the method described by Zlatkis *et al.* (1953) to measure the cholesterol.

Table 2. Changes of body weight and diet intake during feeding of *Pholiota adiposa* (ASI 24018) extracts

	Weight gain (g/day)	Intake (g/day)	FER
NF	0.21 ± 0.02	4.88 ± 0.18	0.04 ± 0.00
HF	0.23 ± 0.04	4.21 ± 0.17	0.05 ± 0.01
HF1	0.21 ± 0.02	4.63 ± 0.25	0.04 ± 0.01
HF2	0.27 ± 0.02	4.65 ± 0.31	0.06 ± 0.00
HF3	0.23 ± 0.03	4.41 ± 0.16	0.05 ± 0.01

NF, HF, HF1, HF2 and HF3 were same as Table 1.
Food efficiency ratio = Body weight gain (g/day)/Food intake (g/day).

Table 3. Changes of organs weight in body during feeding of *Pholiota adiposa* extracts

	Liver (g)	Kidney (g)
NF	1.56 ± 0.03[1,2]	0.53 ± 0.02[3]
HF	2.24 ± 0.33	0.52 ± 0.02
HF1	2.29 ± 0.17	0.50 ± 0.02
HF2	2.33 ± 0.15	0.50 ± 0.02
HF3	2.17 ± 0.11	0.52 ± 0.01

NF, HF, HF1, HF2 and HF3 were same as Table 1.
[1]Data are expressed as Mean S.E. (n = S:7. S+G:7. OVX:8, OVX+G:8).
[2]Values with different alphabet within the same column are significantly different at $P < 0.05$ by Duncan's multiple range test.
[3]NS : Not significant.

Statistical analysis. The data from the animal experiments, which we present as means ± SEM, are analyzed with the aid of a one way analysis of variance. We also analyzed the differences by using Duncan's new multiple-range test (Duncan, 1957). A p value of less than 0.05 was accepted as being a statistically significant difference.

Results and Discussion

Effect on weight gain, intake and organ weights. Table 2 shows the effect of *P. adiposa* extracts on weight gain, intake and organ weight in hyperlipidemic mice. The mice on a high-fat diet show a higher weight gain and lower intake than the mice on a normal-fat diet. Of the groups of mice whose diet was supplemented with a *P. adiposa* extract, the group with the 0.5% *P. adiposa* supplement shows the highest weight gain (0.27 g/day ± 0.02 g/day).

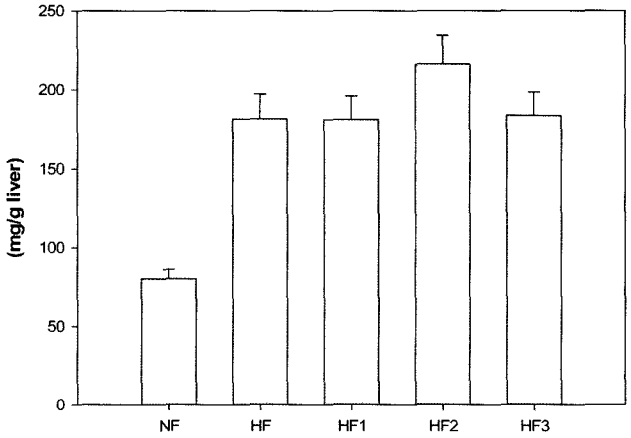

Fig. 1. Changes of total triglyceride contents in the liver during feeding of *Pholiota adiposa* extracts. NF, HF, HF1, HF2 and HF3 were same as Table 1.

Furthermore, as shown in Table 3, the liver weight of the mice on the high-fat diet is heavier than the liver weight of the mice on the normal-fat diet. However, there is no difference in liver weight between the groups whose diet was supplemented with a *P. adiposa* extract. Similarly, there is no difference in kidney weight between all the groups whose diet was supplemented with a *P. adiposa* extract.

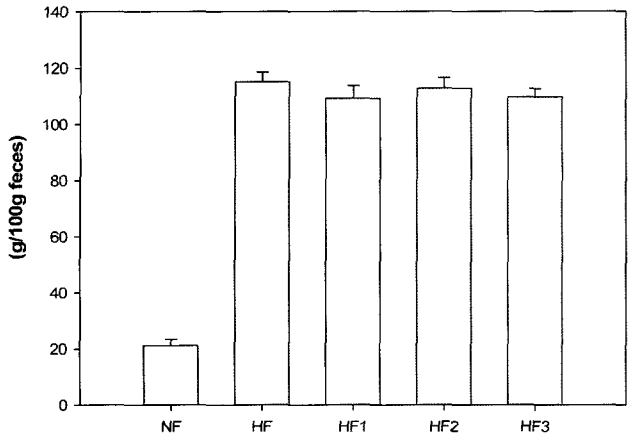

Fig. 2. Changes of total triglyceride contents in the feces during feeding of *Pholiota adiposa* extracts. NF, HF, HF1, HF2 and HF3 were same as Table 1 ($P < 0.05$).

Hepatic and fecal triglyceride. Changes in the total triglyceride contents of the liver were investigated in the various groups whose diet was supplemented with *P. adiposa* extracts (Fig. 1). The total triglyceride contents of the liver is higher in the mice on the high-fat diet than in the mice on the normal-fat diet. However, there is no difference between the groups whose diet was supplemented with a *P. adiposa* extract, except for the group with the 0.5% supplement.

The total triglyceride contents of the feces resembles the total triglyceride contents of the liver (Fig. 2). This results suggests that the *P. adiposa* extract is not affected by a reduction of the triglyceride contents in liver or feces.

Adipose tissue weight. To investigate changes in the retroperitoneal fat mass of mice on a high-fat diet supple-

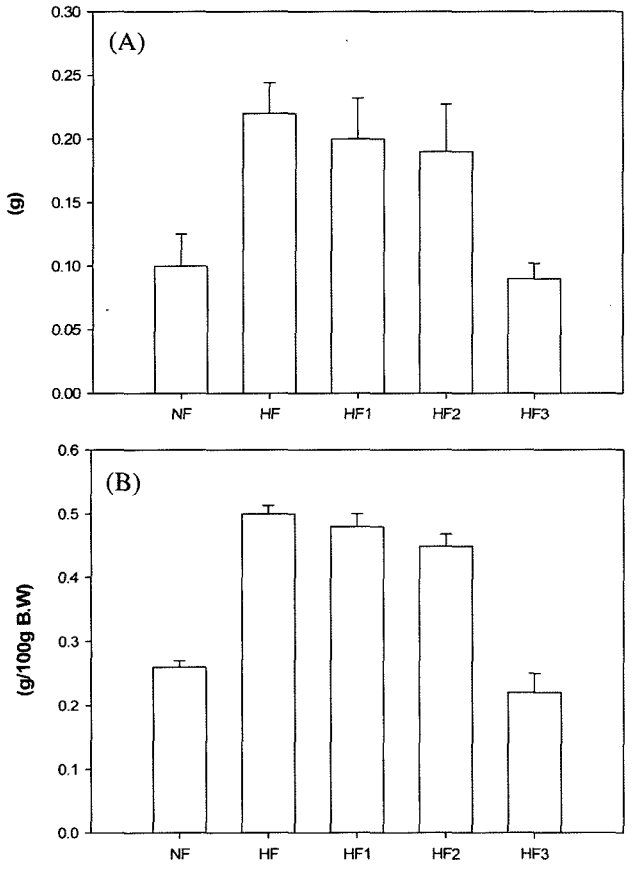

Fig. 3. Changes of fat contents in the kidney periphery during feeding of *Pholiota adiposa* extracts. NF, HF, HF1, HF2 and HF3 were same as Table 1 ($P < 0.05$). A, Fat of kidney periphery; B, Fat of kidney periphery per body weight.

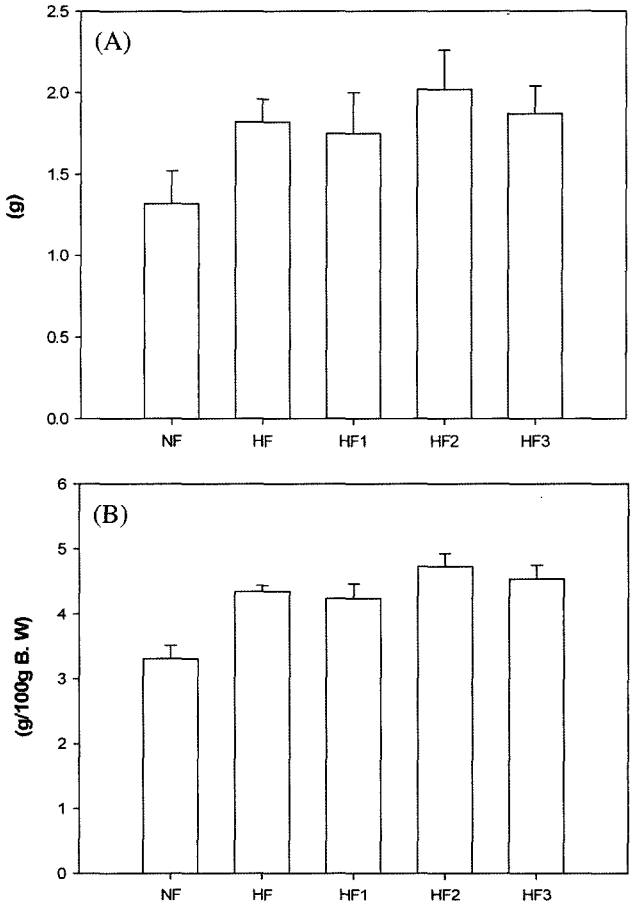

Fig. 4. Changes of fat contents in the epididymis during feeding of *Pholiota adiposa* extracts. NF, HF, HF1, HF2 and HF3 were same as Table 1 ($P < 0.05$). A, Fat of epididymis; B, Fat of epididymis per body weight.

mented with a *P. adiposa* extract, we compared the results with those of mice on a normal-fat diet (Fig. 3). When we increased the *P. adiposa* extracts content, the retroperitoneal fat mass and the retroperitoneal fat mass per body weight in the mice on a high-fat diet decreased to that of mice on a normal-fat diet. This result indicates that *P. adiposa* water extracts may be a potential candidate for use as a functional food that not only acts as a prophytic against hyperlipidemia but also provides health benefits in terms of counteracting hyperlipidemia and related complications.

Meanwhile, the epididymal fat content of the feces was not affected by a diet of *P. adiposa* extracts (Fig. 4).

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