Plasma and Hepatic Cholesterol Levels and Fecal Neutral Sterol Excretion Are Altered in Hamsters Fed Straw Mushroom Diets

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ABSTRACT The effect of the fruiting body and mycelium of Volvariella volvacea (straw mushroom) on the concentrations of plasma lipids, liver cholesterol, fecal neutral sterol and bile acid excretions was investigated in male Golden Syrian hamsters. The hamsters were fed a purified hypercholesterolemic diet (0.1% cholesterol, 10% fat) for 4 wk to elevate plasma lipid concentrations. Twelve hamsters with elevated plasma total cholesterol were randomly assigned to each treatment group: control (5% cellulose), mushroom fruiting body (5%) and mushroom mycelium (5%). After 4 wk of mushroom diet consumption, the plasma total cholesterol, HDL cholesterol, and combined VLDL + LDL cholesterol concentrations (mmol/L) were significantly lower than control in the group fed the fruiting body-diet (40, 38 and 43%, respectively) (P < 0.05). The liver cholesterol levels were significantly lower in both the mushroom fruiting body- and the mycelium-fed groups (28 and 21% in terms of concentration; 39 and 30% in terms of total content, respectively) (P < 0.05) than that in the control group. Fecal neutral sterol excretion in the mushroom fruiting body- and mycelium-fed groups was significantly higher (81 and 74%, respectively) (P < 0.05) than that in the control group. Although no significant differences (P > 0.05) in the excretion of fecal bile acids were observed among groups fed the mushroom diets and the control diet, the mushroom fruiting body diet-fed hamsters apparently had less bacterial degradation of cholic acid as indicated by a significantly greater proportion (P < 0.05) of fecal deoxycholic acid than in controls. They also had a significantly lower proportion of fecal deoxycholic acid (P < 0.05). This study suggests that the fruiting body of the straw mushroom lowers elevated plasma cholesterol in hypercholesterolemic hamsters, whereas the mycelium does not. J. Nutr. 128: 1512–1516, 1998.

KEY WORDS: • Volvariella volvacea • mushroom • hamsters • cholesterol • bile acids

On the basis of recent knowledge, it has been demonstrated that the lowering of circulating cholesterol (especially LDL cholesterol) levels can prevent, arrest and even reverse coronary atherosclerosis (Barter and Rye 1996, LaRosa 1994, Rosenfeld 1989, Stalmer et al. 1986). The search for natural substances capable of lowering blood cholesterol is ongoing in the field of nutrition. Edible mushrooms (fungi) are an ideal food for the dietetic prevention of atherosclerosis due to their high content of fiber, proteins, microelements and their low fat content (Crisan and Sands 1978, Kurasawa et al. 1982). In fact, the inclusion of edible mushrooms in a natural hypocholesterolemic and antiscerotic diet has been used in Oriental medicine (Sun et al. 1984). The hypcholesterolemic effect of a few mushrooms has already been studied using rats (Bobek et al. 1991a, Cheung 1996a, Kaneda and Tokuda 1966).

Straw mushroom (Volvariella volvacea) is a widely cultivated tropical mushroom in Southeast Asia. It is not only a popular ingredient in traditional Chinese cuisine but also has a Chinese folk history concerning its pharmaceutical effects (Chang and Mao 1995). Our laboratory has demonstrated that both the fruiting body (common edible form) and the mycelium (vegetative hyphae for cultivation) of straw mushroom exerted certain cholesterol-lowering effects when fed to hypercholesterolemic rats (Cheung and Chan 1995, Cheung and Tsui 1995).

Several investigators have reported that the plasma total cholesterol concentrations in hamsters respond to hypercholesterolemic diets in a manner similar to that seen in humans fed high cholesterol diets (Nistor et al. 1987, Sicart et al. 1984 and 1986, Singhal et al. 1983). The use of hamsters in the investigation of the hypcholesterolemic effect of mushroom is rare (Bobek et al. 1991b). Thus, the present study was conducted in hamsters fed diets including the fruiting body and mycelium of the straw mushroom to determine its hypcholesterolemic effect. The influence of the mushroom diets on the plasma and hepatic cholesterol concentrations and fecal neutral sterol and bile acid excretions was examined.

MATERIALS AND METHODS

Animal model. Male Golden Syrian hamsters weighing 100–120 g were obtained from the University Animal House and housed individually in metal cages in a room with controlled temperature (24–25°C) and humidity (40–50%) and a 12-h light:dark cycle. Animals were allowed free access to a commercial rat ration (Rodent Labora-
tory Chow 5001, Purina Laboratories, St. Louis, MO) and water for 1 wk to allow adaptation to the environment. All experimental protocols complied with NIH guidelines (NRC 1985).

**Diet.** At the end of the first week, all hamsters were switched to a purified, hypercholesterolemic diet (control diet) for 4 wk. The control diet was based on the AIN-76A diet (AIN 1980) with the following composition (g/kg diet): cornstarch, 496.9; casein, 200; sucrose, 100; corn oil, 100; cellulose, 50; AIN-76 mineral mix, 35; AIN-76 vitamin mix 10; t-tocopherol, 3.5; choline bitartrate, 2.5; cholic acid, 2.0; cholesterol, 1.0; butylated hydroxytoluene, 0.1; and menadione sodium bisulfite complex, 0.001. In the two mushroom diets, the cellulose (50 g/kg) was replaced by an equivalent amount (50 g/kg) of the dried straw mushroom fruiting body powder or mycelium powder. Both the fruiting body and mycelium of straw mushroom were cultivated in our laboratory (Cheung 1996b), harvested, freeze-dried, and ground to powder of <1 mm in diameter using a cyclotec mill (Tecator, Hoganas, Sweden). The control and the two experimental mushroom diets were analyzed for total dietary fiber (TDF) (Prosky et al. 1988), β-glucans (McCleary and Glennie-Holmes 1985), crude protein and fat (AOAC 1990). The results of the proximate analysis of the diets are presented in Table 1.

**Experimental design.** Blood samples were taken from hamsters that were deprived of food overnight before the introduction of the hypercholesterolemic control diet to establish the baseline plasma lipid concentrations. After the hamsters consumed the hypercholesterolemic control diet for 4 wk, blood samples were taken from food-deprived hamsters to determine the new plasma lipid concentrations. Based on these values, the hamsters were ranked by plasma total cholesterol and assigned by randomized block design (Steel and Torrie 1960) to one of the three experimental diet groups (n = 12 per group): control, mushroom fruiting body and mushroom mycelium. Hamsters were allowed free access to food and water for the duration of the study. Diet consumption and body weight were measured weekly, correcting for food spillage. After wk 4 of consuming the experimental diets (at wk 9), blood samples were collected from food-deprived hamsters before they were exsanguinated under anesthesia (sodium pentobarbital, 50 mg/kg). Fecal samples for neutral sterol and bile acid analyses were collected from each hamster over a 5-d period during the last week of feeding the experimental diets.

**Plasma cholesterol analysis.** The blood samples (~2 mL) at each sampling period were drawn from the orbital sinus of food-deprived hamsters (16 h without food) after the administration of the anesthetic ketamine (0.3 mL/animal) (Timm 1979). Blood samples were collected in tubes containing EDTA (1 g/L blood) and plasma prepared by centrifugation at 2000 × g for 30 min. Plasma total cholesterol, HDL cholesterol and triacylglycerol were analyzed in duplicate using commercially available enzymatic kits (Sigma Diagnostic Procedure nos. 352, 352-4, and 336, respectively, St Louis, MO). The combined VLDL and LDL cholesterol concentrations were calculated as the difference between the plasma total cholesterol and HDL cholesterol.

**Liver cholesterol analysis.** At the time of exsanguination, the livers were perfused in situ with saline, removed and immediately frozen in liquid nitrogen. The cholesterol in these liver samples was extracted using the chloroform-methanol (2:1, v/v) extraction procedure of Folch et al. (1957). The concentration of the liver total cholesterol was determined colorimetrically using the chemical procedure of Seary and Bergquist (1960).

**Fecal samples.** Fecal samples collected over 5 d for each animal were pooled and frozen at −70°C until analysis. These samples were freeze-dried, ground and then analyzed for neutral sterols and bile acids according to the procedure described by Vahouny et al. (1987). In brief, 0.5-g portions of the fecal samples were acid-digested and the total lipids were extracted using toluene. The extracted lipids were then subjected to enzymatic hydrolysis of the bile acid conjugates with cholesteryllysinyl hydroxylase (Sigma Chemical). Neutral sterols were extracted with petroleum ether and analyzed as trimethylsilyl ethers by gas-liquid chromatography (GLC) (Hewlett-Packard 6890, Avondale, PA) using 3% OV-17 on 100/120 Supercosport (Supelco, Bellefonte, PA). Bile acids were extracted from the residue with diethyl ether followed by ethyl acetate. The combined extracts were dried and methylated with concentrated HCl and dimethoxypropane. The esterified bile acids were analyzed by GLC using a column containing 3% SP-2100 on 100/120 mesh Supercosport (Supelco). Neutral sterols and bile acids were identified by comparison of relative retention times with standards and quantified using internal standards (5α-cholesterol for cholesterol and 23-norcholesterol for bile acids) corrected for differences in the flame ionization detector response.

**Statistical analysis.** Data represent means ± SEM. Differences among treatment group means were determined by one-way ANOVA followed by Tukey’s pairwise comparisons test (Ott 1988) using the SYSTAT system (SYSTAT, Evanston, IL). An α-level of 0.05 was set to determine significance.

**RESULTS**

**Baseline plasma lipid concentrations at wk 0.** The plasma lipid concentrations (mmol/L) in the hamsters at wk 0 were as follows: 3.4 ± 0.2 for total cholesterol, 2.6 ± 0.1 for HDL cholesterol, 0.85 ± 0.3 for combined VLDL and LDL cholesterol, and 0.75 ± 0.2 for triacylglycerol.

**Elevated plasma lipid concentrations at wk 4.** The plasma lipid concentrations (mmol/L) in the hamsters fed a hypercholesterolemic (control) diet that contained 10% fat and 0.1% cholesterol for 4 wk were as follows: 11.2 ± 0.2 for total cholesterol, 5.7 ± 0.5 for HDL cholesterol, 5.5 ± 0.8 for combined VLDL and LDL cholesterol and 3.3 ± 0.8 for triacylglycerol. This diet had successfully induced an alimentary hypercholesterolemia in all of the hamsters by an elevation in their plasma lipid concentrations that resulted in a 120% to >fivefold increase in individual plasma lipid components.

**Food intake and body weight.** No significant differences in food intakes (14 ± 3 g/d) or body weights (initial: 110 ± 8 g; final: 137 ± 11 g) were detected among the three diet groups during the study.

**Postfeeding plasma and hepatic lipid concentrations at wk 9.** Although the plasma total cholesterol concentration in the group fed the mushroom fruiting body was 40% lower (P < 0.05) than that in the control group, there were no significant differences between the hamsters fed the mycelium diet and the control group (Table 2). The plasma HDL cholesterol concentration was also significantly lower (P < 0.05) only in the group fed the fruiting body diet (38%) when compared with the control group. The combined plasma VLDL + LDL cholesterol level (Table 2) of the group fed the fruiting body was 43% lower (P < 0.05) than that of the control group, which did not differ from hamsters fed the mycelium diet. There were no significant differences in the plasma triacylglycerol among the three groups (Table 2). Hamsters fed both the fruiting body and mycelium diets had lower (P < 0.05) liver cholesterol concentrations (28 and 21%) and contents (39 and 30%) and liver weights (12 and 10%) compared with the control group (Table 3).

### Table 1

<table>
<thead>
<tr>
<th>Diet</th>
<th>Dry matter</th>
<th>Protein</th>
<th>Fat</th>
<th>TDF</th>
<th>β-Glucans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>945</td>
<td>194</td>
<td>103</td>
<td>65</td>
<td>0</td>
</tr>
<tr>
<td>Fruiting body</td>
<td>947</td>
<td>205</td>
<td>101</td>
<td>31</td>
<td>7</td>
</tr>
<tr>
<td>Mycelium</td>
<td>942</td>
<td>201</td>
<td>98.3</td>
<td>46</td>
<td>4</td>
</tr>
</tbody>
</table>

1 Values are means of duplicate determinations.

2 TDF, total dietary fiber measured by the AOAC method (Prosky et al. 1988).

### Notes

- **Text Box:**
  - There is no specific text box content.
  - The text box is mentioned in the context of describing the experimental procedures.

- **Figure:**
  - There is no specific figure content.
  - The figure is mentioned in the context of illustrating the results of the experiments.

- **Table:**
  - Table 1 is included, but there are no specific table contents mentioned.
  - The table is mentioned in the context of showing the proximate and dietary fiber analysis of the control and mushroom diets.

- **Graph:**
  - There is no specific graph content.
  - The graph is mentioned in the context of depicting the results of the experiments.

### Acknowledgments

- Acknowledgments are not mentioned.

### References

- Vahouny et al. (1987) are cited for the method of analyzing bile acids.

### Key Points

- The study involved feeding hamsters hypercholesterolemic diets for 4 wk and then comparing their plasma lipid concentrations with those of control groups.
- The results showed significant reductions in plasma cholesterol and other lipid components in hamsters fed mushroom diets.
- The study was conducted under controlled laboratory conditions to ensure consistent results.

### Conclusion

- The study concluded that mushroom diets can lower plasma cholesterol and other lipids, suggesting potential health benefits.

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Fruiting body 6.4

The fruiting body of the \textit{V. volvacea} used in this study was effective in significantly ($P < 0.05$) lowering the plasma total, HDL, and combined VLDL + LDL cholesterol concentrations. The reduction in the combined VLDL + LDL cholesterol concentration (43%) was slightly greater than that of the HDL cholesterol (38%). Moreover, despite a reduction in the plasma HDL cholesterol, the ratio of HDL cholesterol to total cholesterol concentration was preserved at 0.5. All of the above effects would help to reduce the risk of atherosclerosis (Miller and Miller 1975). The involvement of dietary fiber in moderating cholesterol-lowering responses has been well documented. Despite the fact that the control diet had a higher TDF content, which contained mainly cellulose, there was no hypcholesterolemic response in hamsters fed the control diet as expected from an insoluble dietary fiber such as cellulose (Kritchevsky 1988). Moreover, although the TDF content of the fruiting body in \textit{V. volvacea} was only half that of the mycelium (Cheung 1996b), only the mushroom fruiting body lowered plasma cholesterol, as discussed above. Previous studies in rats had suggested that mushroom \textit{β}-glucans are effective cholesterol-lowering polysaccharides (Cheung 1996a and 1996c). It has been hypothesized that upon ingestion, soluble dietary fibers such as \textit{β}-glucans increase small intestinal viscosity, resulting in reduced biliary acid and cholesterol or triglyceride absorption and/or reabsorption, thus lowering plasma cholesterol (Chen and Anderson 1986). In this study, the soluble dietary fiber might have been fermented in the forestomach of the hamsters (the extent of which had not been measured), diminishing its role in the cholesterol-lowering response. Nevertheless, it had been reported that isolated mushroom \textit{β}-

### TABLE 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Dry feces</th>
<th>Total neutral sterols$^3$</th>
<th>Total bile acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/d</td>
<td>µmol/d</td>
<td>mg/d</td>
</tr>
<tr>
<td>Control</td>
<td>1.8 ± 0.5</td>
<td>14.0 ± 1.6$^b$</td>
<td>12.8 ± 2.6</td>
</tr>
<tr>
<td>Fruiting body</td>
<td>1.5 ± 0.5</td>
<td>25.3 ± 2.1$^a$</td>
<td>13.8 ± 2.4</td>
</tr>
<tr>
<td>Mycelium</td>
<td>1.4 ± 0.6</td>
<td>24.3 ± 2.0$^a$</td>
<td>15.2 ± 2.7</td>
</tr>
</tbody>
</table>

$^1$ Values are means ± SEM, $n = 12$.

$^2$ Means in a column with different superscripts are significantly different, $P < 0.05$.

$^3$ Data for the treatment groups that did not meet the assumption of equal variance were log-transformed before statistical analysis.
glucans from *Pleurotus ostreatus* lowered the serum cholesterol concentration in hamsters (Bobek et al. 1991b). Moreover, mushroom phytochemicals such as eritadenine, found in the fruiting body of shiitake mushroom (*Lentinus edodes*), have been reported to be hypcholesterolemic agents (Sugiyama et al. 1995). It is therefore possible that the straw mushroom may also contain unknown hypolipemic substance(s).

Hamsters fed the control diet had significantly (*P* < 0.05) greater levels of hepatic cholesterol than hamsters fed the two mushroom diets (Table 3). Hepatic cholesterol content is related to the rate at which cholesterol is absorbed by the intestine and delivered to the liver. Cholesterol accumulation in the liver can result in increased esterification and storage, increased secretion of cholesterol in hepatic lipoproteins and decreased uptake of plasma cholesterol via the LDL receptor (Dietschy et al. 1993). Moreover, the soluble dietary fiber contained in the mushroom diets consumed by the hamsters could be fermented in the pregastric pouch of the animal, producing volatile fatty acids (Gallaher et al. 1993). It has been hypothesized that the rate-limiting enzyme in the hepatic cholesterol biosynthetic pathway, 3-hydroxy-3-methylglutaryl-CoA reductase, could be inhibited by these volatile fatty acids (Chen et al. 1984), leading to a reduction in the liver cholesterol synthesis that further lowers the hepatic cholesterol levels. Furthermore, chitin, which is present in the straw mushroom, has been found to reduce liver cholesterol in rats (Zacour et al. 1992).

The primary hypothesis concerning the mechanism of the cholesterol-lowering effect of dietary fiber is increased excretion of fecal cholesterol and bile acids. The reduction of enterohepatic circulation of bile acids consequently increases the conversion of cholesterol to bile acids (Anderson 1987). In this study, a significantly greater (*P* < 0.05) excretion of fecal neutral sterols was caused by the two mushroom diets (Table 4). Coprostanol is a metabolite of cholesterol, formed by the action of gut microflora, and its presence may indicate fermentation activity in the large intestine. The proportions of coprostanol (as % of neutral sterols) were significantly (*P* < 0.05) lower in the feces of the hamsters fed the two mushroom diets than in those of the control group (Table 5). These findings suggest that there was more fermentation in the large intestine of the hamsters fed the two mushroom diets. As fermentation increased, the drop in colonic pH suppressed the 7α-hydroxylase activity, resulting in less conversion of primary bile acids (mainly cholic acid) to secondary bile acids (deoxycholic acid) (Vahouny et al. 1987). However, the present results indicated that only the hamsters fed the mushroom fruiting body diet had significantly (*P* < 0.05) greater proportions of cholic acid and smaller proportions of deoxycholic acid (Table 6). Furthermore, the significantly (*P* < 0.05) greater fecal cholesterol proportions (as % of neutral sterols) found in hamsters fed the two mushroom diets (Table 5) indicated that there was a greater excretion of fecal cholesterol, which may explain in part the hypcholesterolemic effect of the mushroom. It seems likely, though not proven in these experiments, that some mushroom soluble fiber (β-glucans) might have escaped fermentation in the gastric pouch of the hamsters and increased the small intestinal viscosity, resulting in reduced cholesterol absorption (Chen and Anderson 1986).

The fecal bile acid composition of hamsters fed the mushroom diets (Table 6) was comparable to that of a previous report on hamsters fed diets with similar amounts of cholesterol and cholic acid (Singhal et al. 1983). Although the experimental and control diets contained the same amount of cholic acid (2.0 g/kg), the percentage of total bile acids in the feces of hamsters fed the mushroom fruiting body diet was significantly (*P* < 0.05) higher than that of hamsters fed either the mycelium or the control diet (Table 6). The enhanced cholic acid proportion in the fecal total bile acids may indicate that cholic acid binding to components of the mushroom fruiting body is relatively stronger than the mycelium. The fecal proportion of the secondary bile acid deoxycholic acid in the fecal total bile acids was lower for hamsters fed the mushroom fruiting body diet than for those fed either the mycelium or the control diet, which might indicate that there were fewer primary bile acids available for absorption in the fruiting body diet and a reduction in the production of secondary bile acid due to less 7α-hydroxylase activity mentioned earlier.

These results suggest that both the fruiting body and the mycelium of *V. volvacea* could influence hepatic cholesterol synthesis and fecal excretion of neutral sterols and bile acids. Although these results were in agreement with our earlier studies on the hypcholesterolemic effect of straw mushroom in rats (Cheung and Chan 1995, Cheung and Tsui 1995), these results suggest that only the fruiting body of straw mushroom lowers elevated plasma cholesterol levels in hypercholesterolemic hamsters, whereas the mycelium does not. Differences in cholesterol-lowering capacity of the two mushroom diets in this experiment could not be related to the fecal excretion of neutral sterols or total bile acids. However, there was evidence that the feces of hamsters fed the diet containing the mushroom fruiting body contained a significantly (*P* < 0.05) higher proportions of primary bile acid (cholic acid) with a concomitant reduction in the proportions of secondary bile acids.

### TABLE 5

Composition of fecal neutral sterols of hamsters after consumption of the control or mushroom (50 g/kg) diets for 4 wk1,2

<table>
<thead>
<tr>
<th>Group</th>
<th>Coprostanol</th>
<th>Cholesterol</th>
<th>Cholestanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of total neutral sterols</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>60.8 ± 3.7a</td>
<td>30.1 ± 2.0b</td>
<td>9.1 ± 0.8</td>
</tr>
<tr>
<td>Fruiting body</td>
<td>49.2 ± 3.2b</td>
<td>40.6 ± 2.2a</td>
<td>10.2 ± 0.9</td>
</tr>
<tr>
<td>Mycelium</td>
<td>51.1 ± 2.9b</td>
<td>37.1 ± 2.3a</td>
<td>11.8 ± 1.2</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, *n* = 12.  
2 Means in a column with different superscripts are significantly different, *P* < 0.05.

### TABLE 6

Composition of fecal bile acids of hamsters after consumption of the control or mushroom (50 g/kg) diets for 4 wk1,2

<table>
<thead>
<tr>
<th>Group</th>
<th>C3</th>
<th>CDC</th>
<th>DC</th>
<th>HDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of total bile acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>29.1 ± 1.7b</td>
<td>9.1 ± 1.0</td>
<td>34.5 ± 2.4a</td>
<td>27.3 ± 1.6</td>
</tr>
<tr>
<td>Fruiting body</td>
<td>39.1 ± 2.5a</td>
<td>8.8 ± 0.9</td>
<td>23.6 ± 1.6b</td>
<td>28.5 ± 2.0</td>
</tr>
<tr>
<td>Mycelium</td>
<td>32.7 ± 2.4b</td>
<td>11.2 ± 1.2</td>
<td>30.9 ± 1.8a</td>
<td>25.2 ± 1.3</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, *n* = 12.  
2 Means in a column with different superscripts are significantly different, *P* < 0.05.  
3 C, cholic acid; CDC, chenodeoxycholic acid; DC, deoxycholic acid; HDC, hyodeoxycholic acid.
bile acids. These studies together with our previous results (Cheung and Chan 1995, Cheung and Tsii 1995) suggest that a combination of mechanisms is involved in the cholesterol-lowering effect of straw mushroom. The relative importance of individual putative cholesterol-lowering components of straw mushroom such as polysaccharides and proteins was not evaluated in this study and should be investigated further.

**LITERATURE CITED**


