Cholesterol-Lowering Effects of Maitake (*Grifola frondosa*) Fiber, Shiitake (*Lentinus edodes*) Fiber, and Enokitake (*Flammulina velutipes*) Fiber in Rats

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The effects of mushroom fibers on serum cholesterol and hepatic low-density lipoprotein (LDL) receptor mRNA in rats were investigated. Rats were fed a cholesterol-free diet with 50 g/kg cellulose powder (CP), 50 g/kg maitake (*Grifola frondosa*) fiber (MAF), 50 g/kg shiitake (*Lentinus edodes*) fiber (SF), or 50 g/kg enokitake (*Flammulina velutipes*) fiber (EF) for 4 weeks. There were no significant differences in the body weight, food intake, liver weight, cecum weight, and cecum pH among the groups. Cecal acetic acid, butyric acid, and total short-chain fatty acid (SCFA) concentrations in the SF and EF groups were significantly higher than those in the other groups. The serum total cholesterol concentration in the CP group was significantly higher than that in the MAF and EF groups. The very LDL (VLDL) + intermediate-density lipoprotein (IDL) + LDL-cholesterol concentration in the CP group was significantly higher than that in the MAF, SF, and EF groups, whereas the high-density lipoprotein (HDL)-cholesterol concentration in the EF group was significantly lower than that in the other groups at the end of the 4-week feeding period. The hepatic LDL receptor mRNA level in the EF group was significantly higher than that in the CP group. The fecal cholesterol excretion in the MAF, SF, and EF groups was significantly higher than that in the CP group. The results of this study demonstrate that MAF and EF lowered the serum total cholesterol level by enhancement of fecal cholesterol excretion, and in particular, by enhancement of hepatic LDL receptor mRNA in EF group. [Exp Biol Med Vol. 226(8):758–765, 2001]

Key words: rats; mushroom fiber; cholesterol; LDL receptor mRNA

Serum cholesterol concentrations have been reported to be lowered by ingestion of water-soluble fibers such as pectin and oat bran (1). The hypocholesterolemic effect of plant fibers may be due to fiber-induced alterations of intestinal absorption, intestinal or pancreatic hormone secretion, lipoprotein metabolism, bile acid metabolism, or fermentation by-products and their effects on hepatic cholesterol synthesis (2). Sonoyama *et al.* (3) reported that the plasma cholesterol concentration was significantly lower in rats fed sugar beet fiber than in those fed fiber-free or cholestyramine diets, and this difference was due mainly to a lower high-density lipoprotein (HDL) cholesterol concentration. Short-chain fatty acids (SCFA)—particularly propionate, a fermentable metabolite of soluble fibers—may be involved in lowering the serum cholesterol concentration (4).

Dietary cholesterol presumably suppresses hepatic low-density lipoprotein (LDL) receptor activity (5). The rate-limiting enzyme in endogenous sterol biosynthesis is 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, which catalyzes the synthesis of mevalonate (6). The activity of HMG-CoA reductase is also regulated by changes in the exogenous cholesterol concentration (6). Hara *et al.* (7) reported that SCFA suppress cholesterol synthesis in rat liver and intestine. However, they did not determine the effects of dietary fiber on the LDL receptor mRNA and HMG-CoA reductase mRNA concentrations in liver.

Some Bisodiomycotina mushrooms have the ability to lower the serum cholesterol concentration. It is reported that hiratake (*Pleurotus ostreatus*) lowers the serum cholesterol concentration in rats (8), and that the fruiting body of the straw mushroom (*Volvariella volvacea*) lowers elevated plasma cholesterol in hypercholesterolemic hamsters, whereas the mycelium does not (9). These results suggest that the fruiting body of the straw mushroom could influ-

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ence hepatic cholesterol synthesis and fecal excretion of cholesterol (9). Thus, the present study was conducted in rats fed diets including maitake (*Grifola frondosa*) fiber (MAF), shiitake (*Lentinus edodes*) fiber (SF), and enokitake (*Flammulina velutipes*) fiber (EF) to determine their hypocholesterolemic effects. The influence of these mushroom fiber diets on serum lipids, liver lipids, hepatic enzyme activity, and hepatic mRNAs and fecal neutral sterol and bile acid excretions was examined.

Materials and Methods

Animals and Diets. Male F344/DuCrj rats (8 weeks old) were purchased from Charles River Japan, Inc. (Yokohama, Japan). All animals were housed individually in cages on a 12:12-hr light:dark cycle. Temperature and humidity were controlled at 23° ± 1°C and 60% ± 5%, respectively. The rats were divided into four groups of five animals each by randomization. There were no significant differences in body weights and serum total cholesterol concentrations between groups at the start of the experimental period. The composition of the experimental diet was as follows (grams per 100 g): casein, 25; corn oil, 5; fiber, 5; mineral mixture, 3.5 (AIN-76) (10); vitamin mixture, 1 (AIN-76) (10); choline chloride, 0.2; and sucrose to 100. The experimental groups were fed for 4 weeks on one of the following diets that contained 50 g/kg of MAF, SF, and EF. The compositions of MAF, SF, and EF (grams per 100 g) are shown in Table I. Total dietary fiber, insoluble fiber, water-soluble fiber, protein, lipid, carbohydrate, moisture, and ash were determined by AOAC procedures (11). The control group consisted of rats fed 50 g/kg of cellulose. The maitake, shiitake, and enokitake were kindly provided by Mrs. S. Fujiwara (Aibetsu Town Office, Hokkaido, Japan). The rats were allowed free access to experimental diets and water for 4 weeks. Body weight and feed consumption were recorded weekly and every day, respectively. All animal procedures described conformed to the standard principles in the *Guide for the Care and Use of Laboratory Animals* (12).

Analytical Procedures. Blood samples (1 ml) were collected between 0800 and 1000 hr from the jugular veins of fasting rats. The samples were taken into tubes without an anticoagulant. After the samples stood at room temperature for 2 hr, serum was prepared by centrifugation at 1,500*g* for 20 min. At the end of the experimental period of 4 weeks, all fecal excretion during 2 days was collected. Fecal dry weights did not differ among groups. The rats were sacrificed by ether inhalation, and the livers were quickly removed, washed with cold saline (9 g NaCl/l), blotted dry on filter paper, and weighed before freezing for storage.

Table I. Properties of Mushroom Fibers

Components	MAF	SF (g/100 g)	EF
Moisture	3.6	3.6	3.5
Protein	12.1	6.7	5.6
Lipid	1.6	0.8	1.0
Carbohydrate	8.0	17.7	11.1
Ash	3.2	1.7	2.0
Fiber	71.5	69.5	76.8
Insoluble fiber	67.7	64.4	69.9
Water-soluble fiber	3.8	5.1	6.9

Note. Total dietary fiber, insoluble fiber, water-soluble fiber, protein, lipid, carbohydrate, moisture, and ash were determined by AOAC procedures. CP, cellulose powder; MAF, maitake fiber; SF, shiitake fiber; EF, enokitake fiber.

Chemical Analysis. Total cholesterol, HDL-cholesterol, and triglyceride (TG) concentrations in the serum were determined enzymatically using commercially available reagent kits (assay kits for the TDX system; Abbott Laboratory Co., Irving, TX). The very LDL (VLDL) + intermediate-density lipoprotein (IDL) + LDL-cholesterol concentration was calculated as follows: VLDL + IDL + LDL-cholesterol = total cholesterol − HDL-cholesterol.

Total lipids were extracted from liver and feces by a mixture of chloroform/methanol (2:1, v/v) (13). The neutral sterol in each total lipid obtained by saponification was acetylated (14) and analyzed by gas-liquid chromatography (GLC) using a Shimadzu 14A chromatograph (Kyoto, Japan) with a DB17 capillary column (0.25 mm × 30 m; J&W Scientific, Folsom, CA) with nitrogen as the carrier gas. Acidic sterols in feces were measured by GLC following the method of Grundy *et al.* (15). A part of the cecum was taken out into desalting water in a vial without exposure to air, and was suspended. The suspension of the cecum was deproteinized by perchloric acid (final concentration of 50 g/l) cooled in ice, and the supernatant was added to a NaOH solution to precipitate perchloric acid and to form sodium salts of the SCFA. Individual SCFA was measured by GLC with a glass column (2,000 × 3 mm) packed with 80- to 100-mesh chromosorb W-AW DMCS with H_3PO_4 (100 ml/l) as the liquid phase after adding H_3PO_4 by the procedure of Hara *et al.* (16).

Rat Liver Enzyme Preparation. The liver was homogenized in two volumes of cold medium containing 50 m*M* KCl, 2 m*M* $MgCl_2$, 20 m*M* Tris-HCl (pH 7.6), and 250 m*M* sucrose in a Potter-Elvehjem-type homogenizer. After homogenization with only four strokes, the mixture was centrifuged at 1,000*g* for 10 min, and the supernatant fraction was then centrifuged at 12,000*g* for 15 min. The supernatant fraction from this centrifugation was further fractionated by centrifugation at 105,000*g* for 60 min and the resulting pellet was called the microsomal (Ms) fraction. This Ms fraction was washed by centrifugation at 12,000*g* for 15 min and then at 105,000*g* for 60 min in the suspension medium, and was finally suspended in 150 m*M* KCl (pH 7.6) containing 1 m*M* EDTA.

Determination of HMG-CoA Reductase (*EC* 1.1.1.34) Activity. The present procedure followed the method of Lippe *et al.* (17) with some modifications (18). A 1.5-mg sample of protein was suspended in 200 μl of a solution containing 250 m*M* NaCl, 50 m*M* potassium phos-

phate (pH 7.2), 10 m*M* EDTA, and 10 m*M* dithiothreitol (DTT). The sample was pre-incubated for 20 min at 37°C and the reaction was started by adding 25 μl of a solution containing 300 m*M* glucose-6-phosphate, 25 μl 30 m*M* NADP, 1 IU glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49), and 50 μl of 0.14 m*M* [3^{14}-C]HMG-CoA (0.25 MBq/ml, specific activity 59,568 disintegrations/min per nmol). After a 30-min incubation at 37°C, the reaction was stopped with 0.1 ml of 2 *M* HCl and the sample was left for 30 min at 37°C to allow lactonization of mevalonic acid. It was then cooled in ice and centrifuged for 10 min at 3,000*g*. To the supernatant fraction was added 10 μl of 0.5 *M* mevalonolactone (carrier) and 100 mg of $Na_2S_2O_3$. The final pH of the solution was 6.5. After double extraction with 2 ml of benzene, the extract was applied to a silica-gel thin-layer chromatography (TLC) plate and was developed in benzene-acetone (1:1, v/v). The silica gel of the mevalonolactone region, detected with I_2 vapor, was scraped off, transferred to a scintillation vial containing Pico-aqua cocktail (Packard Instrument Co., Inc., Meriden, CT), and the radioactivity measured with a scintillation spectrometer (Packard Instrument Co. Inc., Downers Grove, IL).

Determination of Cholesterol 7α-Hydroxylase (*EC* 1.14.13.17) Activity. The procedure followed is described elsewhere (19). One milligram of microsomal protein was added to a 0.1 *M* potassium phosphate buffer (pH 7.4), 50 m*M* NaF, 5 m*M* DTT, 1 m*M* EDTA, 200 g/l glycerol, and 0.15 g/l 3-([3-cholamidopropyl]-dimethylammonio)-1-propanesulfonate (CHAPS), and the mixture was incubated for 5 min at 37°C. The reaction was initiated by adding NADPH regeneration components, the final concentrations of which were 5 m*M* sodium isocitrate, 5 m*M* $MgCl_2$, 0.5 m*M* NADPH, and 0.075 units of isocitrate dehydrogenase, all in a final reaction volume of 1.0 ml. The reaction mixture was incubated at 37°C for 20 min, unless otherwise indicated, in a shaking (90 strokes/min) water bath. The reaction was terminated by adding 30 μl of 200 g/l sodium cholate, and 1 μg of 7β-hydroxycholesterol as an internal recovery standard. The final reaction was initiated by adding 44 μl of 1 g/l cholesterol oxidase (*EC* 1.1.3.6) in a 10 m*M* potassium phosphate buffer (pH 7.4) containing 1 m*M* DTT and 200 g/l glycerol. This reaction mixture was incubated for 10 min at 37°C, and the reaction terminated by adding 2 ml of 950 g/l ethanol. The cholesterol metabolites from this reaction mixture were extracted by adding 6 ml of petroleum ether, vortexing, incubating at 37°C for 3 min, and then centrifuging (1,200*g*) for 3 min. The upper ethereal layer was collected and dried at 40°C under a nitrogen gas atmosphere. Residues from the various extractions were analyzed by C-18 reverse-phase, high-performance liquid chromatography (Shimadzu 10A) using a Zorbax ODS (4.6 mm × 0.25 mm, 5–6 μm, MAC-MOD Analytical, Inc., Ford, PA) column equilibrated with acetonitrile-methanol (7:3, v/v). The residues were resuspended in 0.1 ml of the same solvent mixture, and 0.02 ml was injected into the column. The metabolites were eluted with the same solvent system at a flow rate of 0.8 ml/min. After 15 min, the flow rate was increased to 2.0 ml/min for 15 min. The amount of product formed was determined by monitoring the absorbance of the effluent at 240 nm and then calculating the number of nanomoles from a calibration curve.

RNA Isolation, RT-PCR, and Southern Blot Analysis. Total RNA was isolated from the liver by the acid guanidium-phenol-choloroform method using Isogen (Nippon Gene, Tokyo, Japan) (20). mRNA encoding apo B, LDL receptor, HMG-CoA reductase, cholesterol 7α-hydroxylase, and GAPDH (used as an invariant control) was analyzed by semi-quantitative RT-PCR and subsequent Southern hybridization of the PCR products with each inner oligonucleotide probe. Total RNA samples were treated with DNase RQ1 (Promega, Madison, WI) to remove genomic DNA and were then subjected to RT-PCR by using Moloney murine leukemia virus reverse transcriptase (Gibco-BRL, Gaithersburg, MD) and EX-Taq polymerase (Takara, Tokyo, Japan) with apo B primers of oligonucleotides (upstream primer, 5′-GAAAGCATGCTGAAAACAACC-3′; downstream primer, 5′-AGGCCTGACTCGTGGAAGAA-3′), LDL receptor primers of oligonucleotides (upstream primer, 5′-ATTTTGGAGGATGAGAAGCAG-3′; downstream primer, 5′-CAGGGCGGGGAGGTGTGAGAA-3′), HMG-CoA reductase primers of oligonucleotides (upstream primer, 5′-GCGTGCAAAGACAATCCTGGAG-3′; downstream primer, 5′-GTTAGACCTTGAGAACCCAATG-3′), cholesterol 7α-hydroxylase of oligonucleotides (upstream primer, 5′-GCCGTCCAAGAAATCAAGCAGT-3′; downstream primer, 5′-TGTGGGCAGCGAGAACAAAGT-3′) , and GAPDH primers of oligonucleotides (upstream primer, 5′-GCCATCAACGACCCCTTCATT-3′, downstream primer, 5′-CGCCTGCTTCACCACCTTCTT-3′). The reaction mixtures for the PCRs contained 25 pmol of each primer, 1.25 U of EX-Taq polymerase, 1 X PCR buffer (Takara), and 200 μ*M* dNTP in a 50-μl reaction volume. The expected sizes of DNA fragments amplified with these primers were 725 bp for apo B, 931 bp for the LDL receptor, 245 bp for HMG-CoA reductase, 306 bp for cholesterol 7α-hydroxylase, and 702 bp for GAPDH. Temperature cycling was as follows: first cycle, denaturation at 94°C for 3 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min. Subsequent cycles were denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min. The thermal cycling was completed by terminal extension at 72°C for 10 min. In total, 25 cycles were performed for the apo B and the LDL receptor amplifications, 30 cycles for HMG-CoA reductase and cholesterol 7α-hydroxylase, and 20 cycles for GAPDH. Amplification products were electrophoresed on 2% agarose gel and transferred to a nylon membrane (Biodyne B, Pall Bio-Support, East Hills, NY). Blots were hybridized with an apo B probe of a 54-base oligonucleotide (5′-TCCTTGCTTACCAAAAAGAGCTTCCAGTGTTGGCTCAAAGCCCTTTCCTTCTAA-3′), LDL receptor probe of

a 54-base oligonucleotide (5′-GTGAACTTGGGTGAG-TGGGCACTGATCTGAGGGGCAGGCAGGCACATG-TACTGG-3′), HMG-CoA reductase probe of a 54-base oligonucleotide (5′-GATCTGTTGTGAACCATGTGACT-TCTGACAAGATGTCCTGCTGCCAATGCTGCC-3′), cholesterol 7α-hydroxylase probe of a 54-base oligonucleotide (5′-CCCGAAGGCCTGTTTAAGTGATGACTCTCA-GCCGCCAAGTGACATCATCCAGTG-3′), and GAPDH probe of a 54-base oligonucleotide (5′-TGATGACCAGC-TTCCCATTCTCAGCCTTGACTGTGCCGTTGAACT-TGCCGTGGG-3′). The probe was 3′-tailing labeled with digoxigenin, using a DIG oligonucleotide tailing kit (Boehringer Mannheim, Germany). Prehybridization, hybridization, and detection were carried out with a DIG luminescent detection kit (Boehringer Mannheim) as recommended by the manufacturer. The relative quantity of mRNA was estimated by densitometry scanning with x-ray film.

Statistical Analysis. Data are presented as means and standard deviations. The mean and standard deviation for serum total cholesterol, HDL-cholesterol, and VLDL + IDL + LDL-cholesterol for each time point were calculated. The significance of differences among treatment groups was determined by analysis of variance with Duncan's multiple-range test (SAS Institute, Cary, NC). Results were considered significant at $P < 0.05$.

Results

There were no significant differences in the body weight gain (CP, MAF, SF, and EF: 71 ± 10, 75 ± 9, 75 ± 10, and 66 ± 7 g/4 weeks, respectively), liver weight (CP, MAF, SF, and EF: 4.0 ± 0.3, 3.9 ± 0.2, 4.0 ± 0.2, and 3.8 ± 0.2 g/100 g body weight, respectively), food intake (CP, MAF, SF, and EF: 407 ± 31, 407 ± 25, 409 ± 12, and 386 ± 40 g/4 weeks, respectively), or cecum weight (CP, MAF, SF, and EF: 1.2 ± 0.3, 1.1 ± 0.2, 1.2 ± 0.2, and 1.2 ± 0.2 g/100 g body weight, respectively) among groups.

Table II shows the serum total cholesterol, VLDL + IDL + LDL-cholesterol, HDL-cholesterol, and TG concentrations of rats. The serum total cholesterol concentration in the CP group was significantly higher than those in the MAF and EF groups, and the VLDL + IDL + LDL-cholesterol concentration in the CP group was significantly higher than those in the MAF, SF, and EF groups at the end of the 4-week feeding period. In particular, the serum total cholesterol concentration in the EF group was the lowest among the groups and the serum VLDL + IDL + LDL-cholesterol concentrations in all treatment groups were significantly lower than that in the CP group throughout the experimental period. The HDL-cholesterol concentration in the EF group was significantly lower than that in the other groups, and there were no significant differences in the TG concentration at the end of the 4-week feeding period.

There were no significant differences in the liver cholesterol concentration (CP, MAF, SF, and EF: 2.65 ± 0.89, 3.17 ± 0.95, 2.86 ± 0.64, and 2.92 ± 0.50 μmol/g wet liver, respectively), HMG-CoA reductase (CP, MAF, SF, and EF: 0.18 ± 0.01, 0.18 ± 0.01, 0.21 ± 0.01, and 0.20 ± 0.02 pmol/min per 1 mg protein, respectively), or cholesterol 7α-hydroxylase (CP, MAF, SF, and EF: 29.5 ± 4.0, 30.8 ± 4.8, 25.6 ± 11.4, and 29.9 ± 11.4 nmol/hr per 1 mg protein, respectively) activities among the groups.

The relative quantities of mRNAs were determined by

Table II. Serum Total Cholesterol, VLDL + IDL + LDL-Cholesterol, HDL-Cholesterol, and Triglyceride Concentrations in Rats Fed Mushroom Fibers for 4 Weeks

Diet group	0 week	1 week	2 weeks (m*M*)	3 weeks	4 weeks
Total cholesterol					
CP	1.68 ± 0.13	2.23 ± 0.35[a]	2.46 ± 0.16[a]	2.51 ± 0.29[a]	2.92 ± 0.18[a]
MAF	1.54 ± 0.12	2.07 ± 0.27[a,b]	2.26 ± 0.18[a]	2.41 ± 0.27[a]	2.59 ± 0.27[b]
SF	1.54 ± 0.13	1.98 ± 0.19[a,b]	2.25 ± 0.10[a]	2.31 ± 0.13[a]	2.63 ± 0.21[a,b]
EF	1.57 ± 0.11	1.79 ± 0.18[b]	1.92 ± 0.16[b]	1.95 ± 0.28[b]	2.19 ± 0.23[c]
VLDL + IDL + LDL-cholesterol					
CP	0.50 ± 0.09	0.84 ± 0.21[a]	0.89 ± 0.15[a]	0.96 ± 0.11[a]	1.07 ± 0.14[a]
MAF	0.40 ± 0.08	0.67 ± 0.14[a,b]	0.69 ± 0.10[b]	0.80 ± 0.14[b]	0.81 ± 0.11[b,c]
SF	0.45 ± 0.06	0.70 ± 0.10[a,b]	0.73 ± 0.05[b]	0.82 ± 0.04[b]	0.85 ± 0.07[b]
EF	0.47 ± 0.07	0.55 ± 0.07[b]	0.61 ± 0.08[b]	0.62 ± 0.10[c]	0.70 ± 0.10[c]
HDL-cholesterol					
CP	1.18 ± 0.04	1.39 ± 0.16	1.57 ± 0.07[a]	1.54 ± 0.20[a,b]	1.85 ± 0.05[a]
MAF	1.13 ± 0.10	1.40 ± 0.17	1.56 ± 0.12[a]	1.61 ± 0.15[a]	1.78 ± 0.17[a]
SF	1.09 ± 0.09	1.28 ± 0.14	1.52 ± 0.07[a]	1.49 ± 0.09[a,b]	1.77 ± 0.15[a]
EF	1.10 ± 0.05	1.24 ± 0.11	1.30 ± 0.11[b]	1.34 ± 0.19[b]	1.49 ± 0.14[b]
Triglyceride					
CP	0.79 ± 0.09	1.34 ± 0.38	1.61 ± 0.15[a,b]	1.89 ± 0.42	2.05 ± 0.43
MAF	0.83 ± 0.20	1.28 ± 0.37	1.64 ± 0.44[a]	1.84 ± 0.44	1.84 ± 0.46
SF	0.72 ± 0.12	1.37 ± 0.26	1.62 ± 0.25[a,b]	1.92 ± 0.28	2.27 ± 0.41
EF	0.75 ± 0.19	1.01 ± 0.15	1.24 ± 0.14[b]	1.45 ± 0.31	1.72 ± 0.39

Note. Values are expressed as means ± SD for five rats. Means within the same columns bearing different superscripts are significantly different ($P < 0.05$). See Table I for abbreviations.

Southern hybridization of PCR-amplified HMG-CoA reductase cDNA (Fig. 1), cholesterol 7α-hydroxylase cDNA (Fig. 1), apo B cDNA (Fig. 2), and LDL receptor cDNA (Fig. 2) in the rat liver. The values of HMG-CoA reductase, cholesterol 7α-hydroxylase, apo B, and LDL receptor mRNAs were normalized to the value of GAPDH mRNA. The values of the mushroom fiber-fed rats were expressed relative to the average values of the CP diet group, which were normalized to 100. The relative quantities of hepatic HMG-CoA reductase mRNA, hepatic cholesterol 7α-hydroxylase mRNA, and hepatic apolipoprotein B mRNA were unaffected by the diets (Figs. 1 and 2). The relative quantity of hepatic LDL receptor mRNA in the EF group was significantly higher than that in the CP group ($P < 0.05$). The relative quantity of hepatic LDL receptor mRNA in the MAF group also tended to be higher than that in the CP group. However, there was no correlation between hepatic LDL receptor mRNA and serum VLDL + IDL + LDL-cholesterol ($r = -0.445$, $0.05 < P < 0.1$).

Table III shows the neutral steroid and acidic steroid concentrations in the feces of rats. There were no significant differences in the fecal total bile acid content among the groups. The fecal cholesterol concentrations in the MAF, SF, and EF groups were significantly higher than that in the CP group.

The SCFA level and pH in the cecum at the end of the experimental period are shown in Table IV. Acetic acid, butyric acid, and total SCFA concentrations in the SF and EF groups were significantly higher than those in the other groups. The propionic acid concentration in the SF group was significantly higher than in the CP group. There was no significant difference in the cecal pH among groups.

Discussion

In the present study we examined the effects of mushroom fibers on serum cholesterol, hepatic LDL receptor mRNA levels, and fecal cholesterol excretion in rats. The serum total cholesterol concentrations in the MAF and EF groups were reduced by 11% to 25% compared with the CP group. Total cholesterol concentrations were reduced in both treatment groups due to lowering of VLDL + IDL + LDL cholesterol, and also to lowering of the HDL cholesterol concentration in the EF group. Sonoyama *et al.* (3). reported that sugar beet fiber reduces ileal concentrations of apo A-I and apo A-IV mRNA in rats. It may be speculated that the effect of EF is due to its lowering action on ileal concentrations of apo A-I and apo A-IV mRNA or its enhancing action on HDL receptor activity in adrenals/gonads. In the present study there were no significant differences in the hepatic concentration of apo B mRNA among the groups. On the other hand, the LDL receptor mRNA level in the EF group was significantly higher than in the CP group and, in the MAF group, it also tended to be higher than in

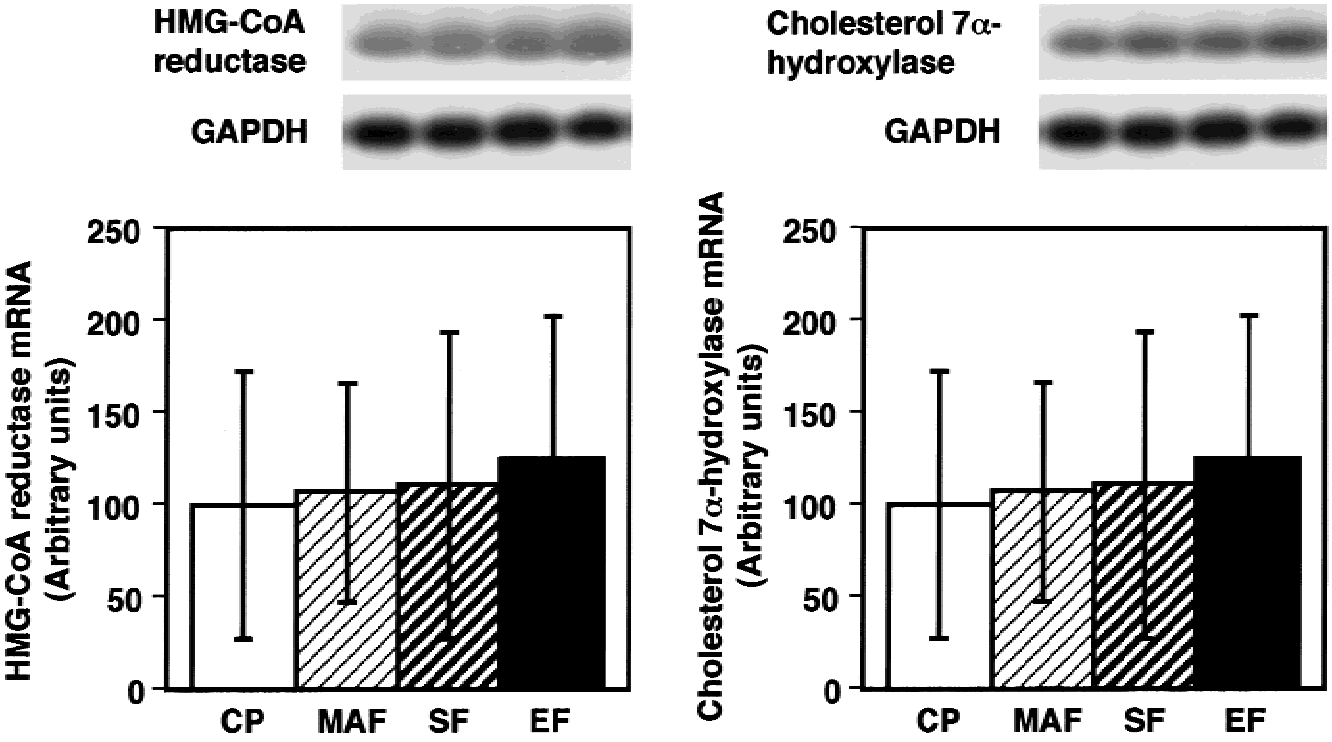

Figure 1. Hepatic HMG-CoA reductase mRNA and cholesterol 7α-hydroxylase mRNA concentrations in rats fed mushroom fibers for 4 weeks. Each value represents the mean ± standard deviations for data obtained from five animals. The value of HMG-CoA reductase and cholesterol 7α-hydroxylase mRNA was normalized to the value of GAPDH, and values for the rats fed the MAF, SF, and EF diets are expressed relative to the average values for rats fed the CP diet, which was set to 100. Inset illustrates the representative Southern hybridization of PCR amplified HMG-CoA reductase cDNA and cholesterol 7α-hydroxylase cDNA of hepatic RNA. CP, cellulose powder; MAF, maitake fiber; SF, shiitake fiber; EF, enokitake fiber.

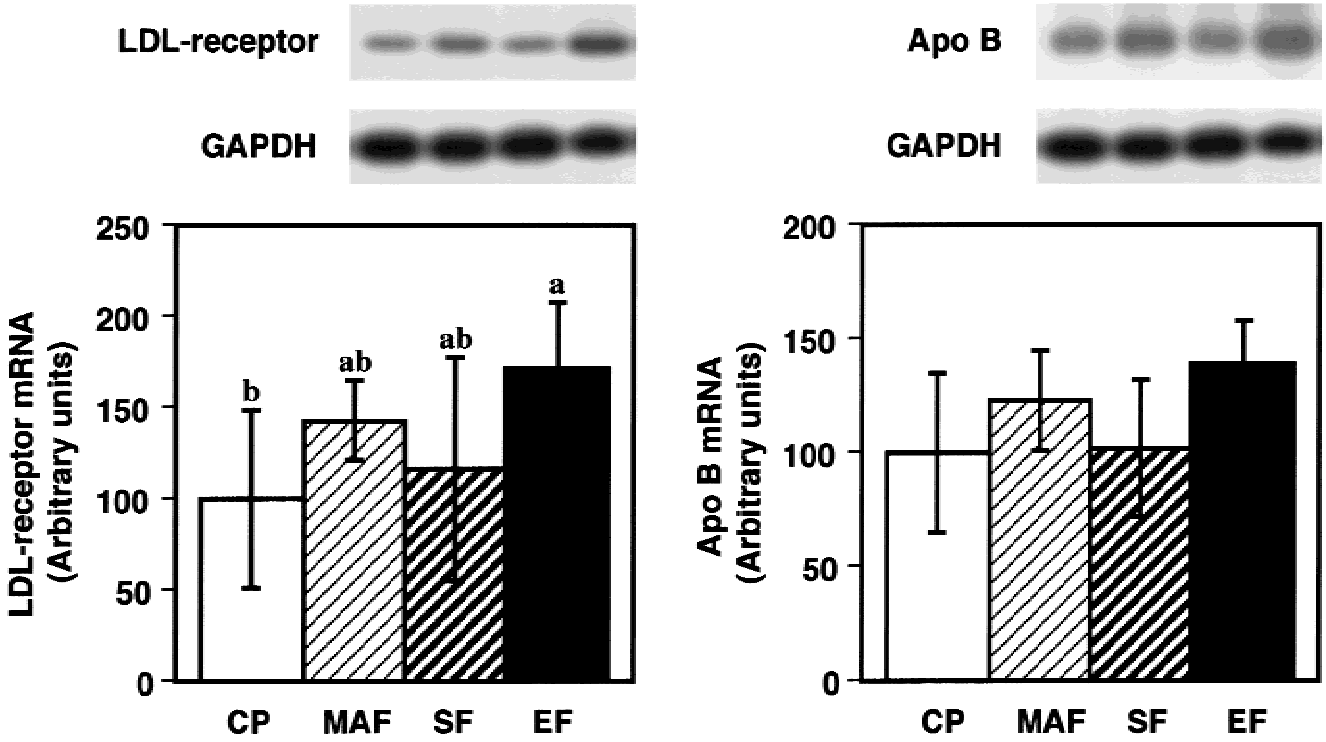

Figure 2. Hepatic apo B mRNA and LDL receptor mRNA concentrations in rats fed mushroom fibers for 4 weeks. Each value represents the mean ± standard deviations for data obtained from five animals. Means values were significantly different ($P < 0.05$), as determined by analysis of variance with Duncan's multiple-range test. The value of apo B and LDL receptor mRNA was normalized to the value of GAPDH, and values for the rats fed the MAF, SF, and EF diets are expressed relative to the average values for rats fed the CP diet, which was set to 100. Inset illustrates the representative Southern hybridization of PCR amplified apo B cDNA and LDL receptor cDNA of hepatic RNA. CP, cellulose powder; MAF, maitake fiber; SF, shiitake fiber; EF, enokitake fiber.

the CP group. One of the reasons for the lower serum VLDL + IDL + LDL-cholesterol concentration in the EF group may have been elevation of the LDL receptor level. We previously reported (21) that the hepatic LDL receptor mRNA level in rats fed a mushroom (*Agaricus bisporus*) fiber was significantly higher than that in rats fed cellulose, and the hepatic LDL receptor mRNA level correlated negatively with the serum VLDL + IDL + LDL cholesterol concentration. In the present experiment we used a cholesterol-free diet to eliminate the influence of exogenous cholesterol. The elevation of the hepatic LDL receptor mRNA level observed in rats fed EF is of particular interest.

There were no significant differences in the liver cholesterol concentration, HMG-CoA reductase activity, and

Table III. Fecal Steroid Concentrations in Rats Fed Mushroom Fibers for 4 Weeks

Component	Diet group			
	CP	MAF	SF	EF
	(μmol/g wet feces)			
Cholesterol	3.03 ± 1.23^{b}	5.49 ± 2.47^{a}	7.58 ± 1.69^{a}	5.95 ± 1.38^{a}
Coprostanol	1.05 ± 0.84^{a}	0.23 ± 0.12^{b}	0.32 ± 0.14^{b}	0.55 ± 0.34^{a,b}
LCA	0.18 ± 0.18	0.21 ± 0.05	0.11 ± 0.04	0.24 ± 0.12
DCA	0.40 ± 0.41	0.32 ± 0.13	0.22 ± 0.05	0.41 ± 0.12
CDCA	0.08 ± 0.06	0.04 ± 0.01	0.04 ± 0.02	0.08 ± 0.04
CA	0.30 ± 0.30	0.12 ± 0.07	0.12 ± 0.06	0.14 ± 0.12
TBA	0.95 ± 0.89	0.69 ± 0.20	0.50 ± 0.18	0.87 ± 0.32
Ratio of primary/secondary bile acid[1]	0.65 ± 0.11^{a}	0.30 ± 0.04^{b}	0.48 ± 0.04^{a,b}	0.35 ± 0.08^{b}
Ratio of sterol/total bile acid[2]	5.11 ± 1.35^{b}	9.16 ± 2.78^{b}	16.90 ± 2.29^{a}	8.46 ± 1.71^{b}

Note. Values are expressed as means ± SD for five rats. Means within the same rows bearing different superscripts are significantly different ($P < 0.05$). LCA, lithocholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; CA, cholic acid; TBA, total bile acid. See Table I for other abbreviations.

[1] Ratio of primary/secondary bile acid, (CA + CDCA)/(DCA + LCA).

[2] Ratio of sterol/total bile acid, neutral sterol/total bile acid.

Table IV. Cecal Short-Chain Fatty Acid Concentrations and pH in Rats Fed Mushroom Fibers for 4 Weeks

Diet group	Acetic acid	Propionic acid	Butyric acid	Total[1]	Cecal pH
	(μmol/g cecal content)				
CP	32.8 ± 6.0^{b}	5.4 ± 1.0^{b}	4.4 ± 1.4^{b}	42.6 ± 7.7^{b}	7.86 ± 0.18
MAF	37.5 ± 8.3^{b}	7.2 ± 2.1^{a,b}	5.4 ± 2.5^{b}	50.1 ± 12.6^{b}	7.70 ± 0.11
SF	56.4 ± 13.2^{a}	8.5 ± 2.2^{a}	11.5 ± 2.4^{a}	76.3 ± 12.6^{a}	7.57 ± 0.47
EF	53.9 ± 11.2^{a}	7.5 ± 1.5^{a,b}	10.6 ± 1.9^{a}	72.0 ± 14.1^{a}	7.56 ± 0.31

Note. Values are expressed as means ± SD for five rats. Means within the same columns bearing different superscripts are significantly different ($P < 0.05$). See Table 1 for abbreviations.
[1] Total = Acetic acid + propionic acid + butyric acid.

HMG-CoA reductase mRNA level among the groups. Hara *et al.* (22) reported that products of fermentation of sugar beet fiber by cecal bacteria lower the plasma cholesterol concentration in rats and that SCFA, as a fermentation product, suppresses cholesterol synthesis in the rat liver and intestine (7). On the other hand, it has been reported that dietary fiber and the SCFA production elevate hepatic cholesterol synthesis (23–26). Although the SCFA concentration was elevated in the cecum in rats fed EF in this study, no effect of elevated SCFA on cholesterol synthesis in the rat liver was demonstrated. Illman and Topping (27) reported that raising the cecal propionate concentration stimulates hepatic cholesterol synthesis through increasing fecal steroid excretion. Illman *et al.* (28) also reported that cecal propionate correlates negatively with the plasma cholesterol concentration and positively with cecal neutral steroids and bile acids. In our study, there was no correlation between cecal SCFA and serum total cholesterol or fecal bile acid ($r = -0.406$, $0.05 < P < 0.1$; $r = -0.184$, $P > 0.1$, respectively). However, the cecal SCFA correlated positively with fecal cholesterol ($r = 0.643$, $P < 0.01$). Kubo and Nanba (29) reported that the antihyperlipemic activities of maitake were due to the acceleration of cholesterol and bile acid excretion, and of cholesterol conversion into bile acid. On the other hand, Cheung (9) reported that straw mushroom diet lowered the plasma and hepatic cholesterol levels and increased fecal neutral sterol excretion in hamsters. We also observed that fecal cholesterol excretion in the MAF, SF, and EF groups agreed with the above report. However, we did not observe significant differences in the fecal total bile acid excretion among the groups. Thus, the lowered cholesterol concentrations in the MAF and EF groups resulted from enhanced fecal cholesterol excretion.

The ratio of primary/secondary bile acids was much higher in the CP group, suggesting lower metabolic conversion compared with the treatment groups (Table III). Buhman *et al.* (30) have reported that feeding psyllium to rats enhances fecal bile acid and total steroid excretion, as well as cholesterol 7α-hydroxylase activity and cholesterol 7α-hydroxylase mRNA levels. In contrast, mushroom fibers did not accelerate cholesterol conversion to bile acid, cholesterol 7α-hydroxylase activity, or the level of cholesterol 7α-hydroxylase mRNA.

The basis for elevated fecal cholesterol was unclear in the present experiment. However, the ratio of sterols/total bile acids was very high in the SF group and tended to increase in the MAF and EF groups as compared with the CP group (Table III). The amounts of insoluble and soluble fibers and differences in transit times in the intestine may have contributed to the observations.

In conclusion, the serum cholesterol-lowering effects in rats of the mushroom fibers were evident compared with rats fed cellulose. The fibers elevated the hepatic LDL receptor mRNA level in the EF group, reduced the HDL-cholesterol concentration in the EF group, and enhanced the fecal cholesterol excretion in the MAF and EF groups. The results demonstrate that ingestion of MAF and EF attenuates increases in the serum total cholesterol and VLDL + IDL + LDL-cholesterol concentrations in test rats.

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