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Note

Effects of *Lentinus edodes* on Fatty Acid and Molecular Species Profiles of Phosphatidylcholine in Rats Fed Different Levels of Corn Oil

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***Shiitake* mushrooms (*Lentinus edodes*) are a hypocholesterolemic and affect phospholipid and fatty acid metabolism in rats. In this study, the effects of 2% shiitake in the diet on fatty acid and molecular species profiles of liver microsomal and plasma phosphatidylcholine (PC) were investigated in rats fed diets containing different levels (1–20%) of corn oil, a linoleic-acid-rich fat. The proportion of 18:2n-6 in PC increased depending on the parcent corn oil, and *L. edodes* further increased the proportion at all corn oil levels. The proportion of 20:4n-6 was lower in rats fed *L. edodes* than in rats fed control diets irrespective of the parcent corn oil. *L. edodes* selectively increased the proportion of 16:0–18:2 molecular species and decreased the proportion of 18:0–20:4 molecular species in PC. These results indicate that the effects of *L. edodes* on fatty acid and molecular species profiles of PC are stronger than that of the dietary corn oil level.**

Key words: *Lentinus edodes*; eritadenine; phosphatidylcholine; corn oil; linoleic acid metabolism

The mushroom *Lentinus edodes* (*shiitake* in Japanese) has hypocholesterolemic effects in rats,[1] caused by eritadenine [2(*R*),3(*R*)-dihydroxy-4-(9-adenyl)-butyric acid].[2,3] In addition to these effects, *L. edodes*[4] and eritadenine[5,6] decrease the ratio of phosphatidylcholine (PC) to phosphatidylethanolamine (PE) in rat liver microsomes, because of decreased PC synthesis *via* the PE *N*-methylation pathway.[5] This decreased ratio suggests that dietary supplementation with *L. edodes* or eritadenine increases the demand for dietary choline, the precursor of PC synthesis *via* the CDP-choline pathway, to compensate for decreased PC synthesis. In support of this, eritadenine-induced fatty liver can be prevented by a high enough dietary choline level.[6] Eritadenine suppresses the metabolism of linoleic acid into arachidonic acid, leading to modifications of fatty acid and molecular species profiles of plasma and liver microsomal phospholipids in rats.[7–9] To sum up, eritadenine affects several aspects of lipid metabolism. Unfortunately, little information is available about the effects of eritadenine on fatty acid metabolism under various dietary conditions, especially the quantity and quality of dietary fat.

Here, we investigated the effects of dietary supplementation with *L. edodes* on the fatty acid and molecular species composition of liver microsomal and plasma PC in rats fed diets containing different levels of corn oil, to examine the relationship between the dietary linoleic acid level and the effect of eritadenine.

Six-week-old male rats of the Wistar strain were obtained from Japan SLC (Hamamatsu, Japan). The rats were housed individually in hanging wire cages of stainless steel and kept in an isolated room at a controlled room temperature (23–25°C) and humidity (50–60%). Lighting was maintained on a 12-h light-dark cycle (lights on from 0600 to 1800 h). Animals were acclimated to the facility and given free access to water and stock diet (Type MF; Oriental Yeast, Tokyo, Japan) for 5 d, and then they were given free access to water and experimental diets for 14 d. The experimental diets contained (g/100 g): casein, 25; sucrose, 20; corn oil, 1, 2, 5, 10 or 20; mineral mixture (AIN-76), 3.5; vitamin mixture (AIN-76), 1; choline chloride, 0.4; cellulose, 2; and corn starch to give 100 g. *Shitake* mushrooms (*L. edodes*), purchased from a market (Shizuoka, Japan) were lyophilized, powdered in a mixer, and added to the feed at the level of 2% to replace cellulose. The amount added was almost high enough to elicit the maximum hypocholesterolemic effect (unpublished data). The fatty acid composition of the corn oil used was as follows (weight %): 16:0, 12.2; 18:0, 1.8; 18:1n-9, 32.8; 18:2n-6, 51.4; 18:3n-3, 1.6. The experimental design was approved by the Laboratory Animal Care Committee of the Faculty of Agriculture at Shizuoka University.

Rats were killed by decapitation between 1100 and 1200 without being starved first. Blood plasma was obtained from heparinized whole blood by centrifugation at 2,000 × *g* for 20 min at 4°C. A portion of the plasma was stored at 4°C until asseyed for plas-

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ma lipid concentrations, and the remainder was stored at −80°C until assayed for fatty acid and molecular species composition of PC. The whole liver was quickly removed, rinsed in ice-cold saline, weighed, and homogenized in 4 volumes of 10 mM Tris-HCl buffer containing 0.25 M sucrose. A portion of the homogenate was centrifuged at 10,000 × *g* for 10 min at 4°C, and the resulting supernatant was centrifuged at 105,000 × *g* for 60 min at 4°C to obtain the microsomal fraction as the precipitate.

The plasma concentrations of total cholesterol, HDL cholesterol, free cholesterol, triglycerides, and phospholipids were measured enzymatically with kits (Cholesterol C-Test, HDL Cholesterol-Test, Free Cholesterol C-Test, Triglyceride G-Test, and Phospholipid B-Test; Wako Pure Chemical Industries, Osaka, Japan). The difference between total cholesterol and HDL cholesterol was taken to be (VLDL + LDL)-cholesterol and that between total cholesterol and free cholesterol was taken to be the cholesteryl esters. The total lipids of liver microsomes and plasma were extracted by the method of Folch *et al.*[10] For assays of fatty acid and molecular species composition, PC of liver microsomes and plasma was separated by TLC on silica gel 60 (E. Merck, Darmstadt, Germany) with chloroform, methanol, and water (65:25:4 by volume), stained with dichlorofluorescein, scraped off the plate, and extracted with chloroform/methanol (1:2 by volume). A portion of PC was treated with 14% (wt/wt) BF_3 and methanol reagent (Wako), and the fatty acid methyl esters formed were analyzed by GLC on a Model GC-17A (Shimadzu, Kyoto, Japan), equipped with a TC-FFAP capillary column (0.25 mm × 30 m; GL Sciences, Tokyo, Japan). Another portion of PC was converted to diacylglycerol benzoates by the method of Blank *et al.*[11] The diacylglycerol benzoates were analyzed by HPLC on a Model LC-6A apparatus (Shimadzu), equipped with an ODS column (4.6 × 250 mm, Lichrosorb RP-18; E. Merck), by the method of Blank *et al.*[11] Liver microsomal protein was measured as described by Lowry *et al.*[12] Results were evaluated by one-way analysis of variance, and the difference between means was examined by Duncan's multiple range test.[13] In some cases, Student's *t*-test was used to examine the significance of differences between the two experimental groups.

Dietary supplementation with 2% *L. edodes* mushroom did not affect the growth, food intake, or relative liver weight compared with the corresponding control groups (data not shown). The concentrations of plasma total cholesterol, (VLDL + LDL)-cholesterol, HDL cholesterol, and cholesteryl esters were lower in rats fed *L. edodes* than in rats fed the corresponding control diets, whatever the dietary corn oil level (Fig. 1). The concentration of plasma phospholipids, but not

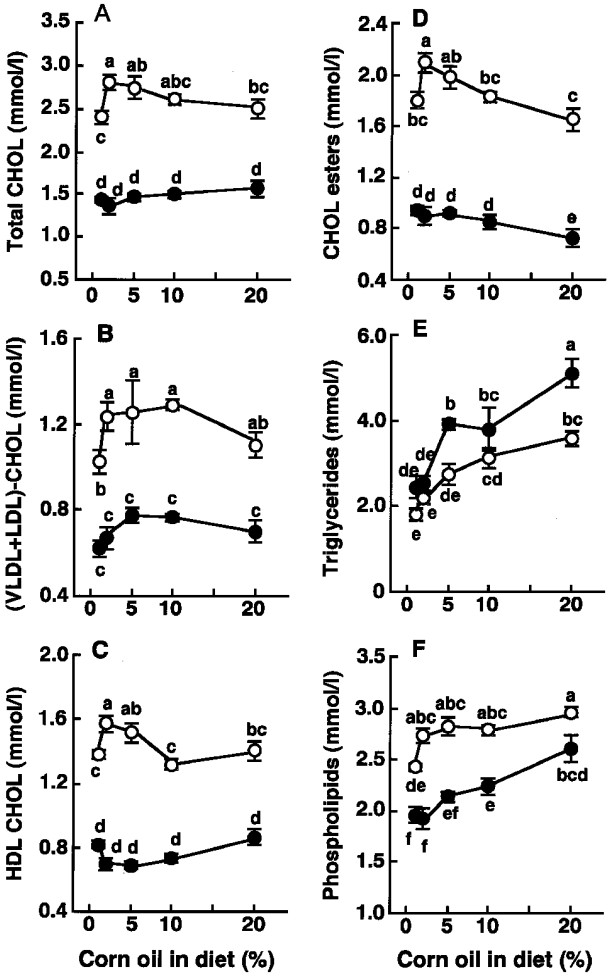

Fig. 1. Effects of Dietary *L. edodes* (2%) on Plasma Lipid Concentrations in Rats Fed Different Levels of Corn Oil (1–20%).

Values are means ± SEM for 6 rats. Values not sharing the same letters are significantly different at $p < 0.05$, Duncan's multiple range test. CHOL, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

○, rats fed control diets; ●, rats fed *L. edodes*-supplemented diets.

triglycerides, also was decreased by *L. edodes*. *L. edodes* did not cause large changes in the liver cholesterol concentration (data not shown). Table 1 shows the effects of dietary *L. edodes* on the fatty acid composition of liver microsomal and plasma PC in rats fed a diet containing a standard level (5%) of corn oil. Almost all of the fatty acids of both microsomal and plasma PC were affected by *L. edodes*, with the increase in 18:2n-6 (linoleic acid) and the decrease in 20:4n-6 (arachidonic acid) especially large. Accordingly, the 20:4n-6/18:2n-6 ratio was decreased by mushroom ingestion to less than half of the control value. The proportion of 22:6n-3 also was decreased by *L. edodes*. Although the proportion of 18:2n-6 in liver microsomal and plasma PC changed depending on the dietary corn oil

Table 1. Effects of Dietary *L. edodes* on Fatty Acid Composition (Weight %) of Liver Microsomal and Plamsa Phosphatidylcholine in Rats Fed a Diet Containing 5% Corn Oil[1]

Fatty acid	Microsomal PC		Plasma PC	
	Control	+ *L. edodes*	Control	+ *L. edodes*
16:0	20.8±0.4	25.6±0.7[2*]	23.8±0.6	28.8±0.4[2*]
16:1n-7	2.9±0.1	3.9±0.3[2*]	1.3±0.1	2.2±0.1[2*]
18:0	17.4±0.5	12.9±0.2[2*]	18.8±0.5	14.0±0.2[2*]
18:1n-9	4.8±0.1	6.0±0.2[2*]	4.6±0.1	6.0±0.3[2*]
18:1n-7	6.2±0.1	5.9±0.2*	4.5±0.2	4.8±0.3[NS]
18:2n-6	13.2±0.5	23.3±0.2[2*]	16.8±0.7	25.6±0.3[2*]
20:4n-6	26.7±0.3	16.7±0.6[2*]	23.0±0.7	13.4±0.4[2*]
22:5n-6	1.5±0.1	0.3±0.0[2*]	1.5±0.1	0.3±0.0[2*]
22:5n-3	0.4±0.0	0.3±0.0[2*]	0.3±0.0	0.3±0.0[NS]
22:6n-3	3.9±0.2	1.9±0.1[2*]	3.1±0.1	1.6±0.1[2*]
20:4n-6/18:2n-6	2.02±0.02	0.72±0.03[2*]	1.36±0.01	0.52±0.02[2*]

[1] Values are means±SEM for 6 rats. *$p<0.05$; [2]*$p<0.001$; NS, not significant. PC, phosphatidylcholine.

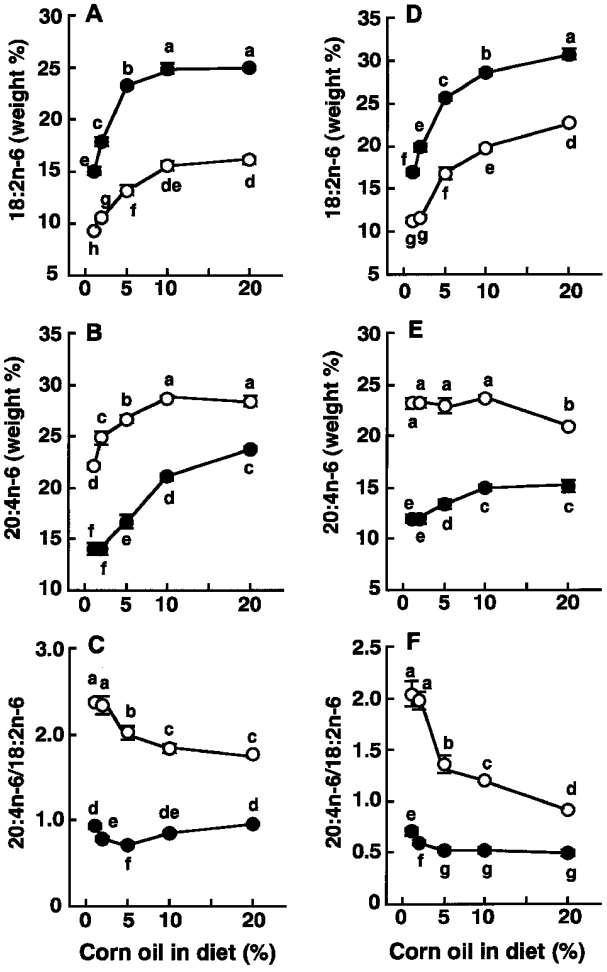

Fig. 2. Effects of Dietary *L. edodes* (2%) on the Proportion of 18:2n-6 and 20:4n-6 and the 20:4n-6/18:2n-6 Ratio of Liver Microsomal (A-C) and Plasma (D-F) Phosphatidylcholine in Rats Fed Different Levels of Corn Oil (1-20%).

Values are means±SEM for 6 rats. Values not sharing the same letters are significantly different at $p<0.05$.

○, rats fed control diets; ●, rats fed *L. edodes*-supplemented diets.

level, the addition of *L. edodes* further increased the proportion of 18:2n-6 at all the such levels (Fig. 2). However, the proportion of 20:4n-6 and the 20:4n-6/18:2n-6 ratio were lower in rats fed *L. edodes* than in control rats, whatever the corn oil level. Table 2 shows the effects of *L. edodes* on the molecular species of liver microsomal and plasma PC in rats fed a diet containing 5% corn oil. All molecular species were affected, more or less, by *L. edodes*, the greatest changes were the increase in the 16:0–18:2 molecular species and the decrease in the 18:0–20:4 molecular species in both liver microsomal and plasma PC. The effects of the corn oil level on the proportion of 16:0–18:2, unlike the proportion of 18:2n-6, were small (Fig. 3). The proportion of 16:0–18:2 molecular species was higher in rats fed *L. edodes* than in control rats, and conversely the proportion of 18:0–20:4 was lower in rats fed *L. edodes* than in rats fed control diets, whatever the dietary corn oil level.

The conversion of linoleic acid into arachidonic acid is important quantitatively and qualitatively in the metabolism of fatty acids, and changes in linoleic acid metabolism are generally reflected in the fatty acid profile of PC, the main class of phospholipids. Therefore, we focused on the effects of dietary *L. edodes* on the fatty acid and molecular species profiles of PC. The 20:4n-6/18:2n-6 ratio seems to be an index of the extent of the conversion of linoleic acid into arachidonic acid, so our results indicate that dietary supplementation with *L. edodes* at the 2% level suppressed the metabolism of linoleic acid regardless of the amount of linoleic acid ingested. We did not measure the activity of Δ6-desaturase, the rate-limiting enzyme of linoleic acid metabolism, but the suppression of linoleic acid metabolism by *L. edodes* might occur because of decreased enzyme activity, as evidenced by the effects of eritadenine.[9] One interesting finding of this study is that suppression of linoleic acid metabolism by *L. edodes* is more effective than increased ingestion of linoleic acid in increasing the

Table 2. Effects of Dietary *L. edodes* on Molecular Species Composition (Mol %) of Liver Microsomal and Plasma Phosphatidylcholine in Rats Fed a Diet Containing 5% Corn Oil[1]

Molecular species	Microsomal PC		Plasma PC	
	Control	+ *L. edodes*	Control	+ *L. edodes*
16:0–18:1	7.5±0.2	10.8±0.3[2*]	7.5±0.2	10.6±0.2[2*]
18:0–18:1	1.0±0.0	1.3±0.0[2*]	1.5±0.0	1.7±0.0[2*]
18:1–18:1	0.4±0.0	1.1±0.1[2*]	0.9±0.2	1.5±0.2[2*]
16:0–18:2	11.3±0.3	22.1±0.5[2*]	18.6±0.6	32.0±0.7[2*]
18:0–18:2	5.9±0.2	8.2±0.2[2*]	11.0±0.3	14.1±0.2[2*]
18:1–18:2	5.1±0.1	7.2±0.1[2*]	3.8±0.1	5.5±0.1[2*]
16:0–20:4	19.6±0.3	14.6±0.2[2*]	18.5±0.4	13.0±0.2[2*]
18:0–20:4	24.7±0.3	13.5±0.4[2*]	21.8±0.5	10.2±0.3[2*]
18:1–20:4	5.4±0.2	4.4±0.0[2*]	4.0±0.1	3.2±0.2[2*]
16:0–22:5	1.4±0.0	1.0±0.0[2*]	1.3±0.0	0.9±0.1[2*]
18:0–22:5	1.8±0.0	1.0±0.0[2*]	1.5±0.0	0.7±0.0[2*]
16:0–22:6	6.3±0.1	2.7±0.0[2*]	4.7±0.1	2.9±0.1[2*]
18:0–22:6	3.8±0.1	1.8±0.0[2*]	2.7±0.0	1.5±0.0[2*]

[1] Values are means±SEM for 6 rats. [2*] $p<0.001$.
[2] Fatty acids in the *sn*-1 and *sn*-2 positions are indicated at the left and right side, respectively. PC, phosphatidylcholine.

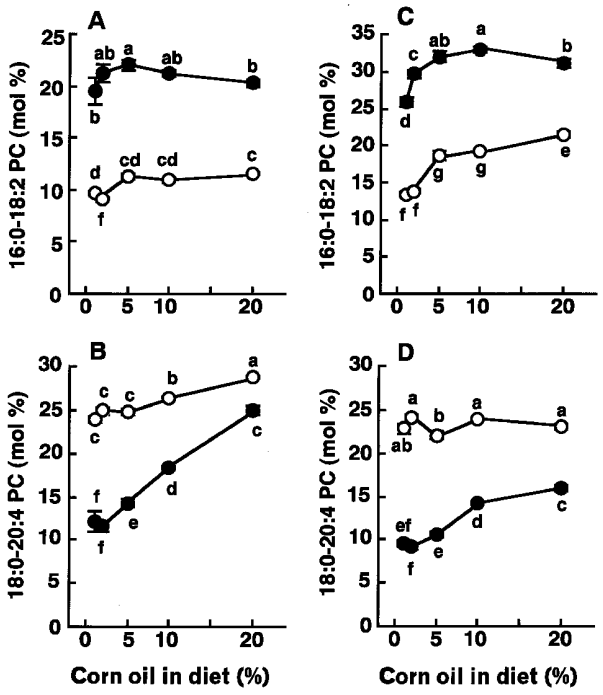

Fig. 3. Effects Dietary *L. edodes* (2%) on the Proportion of Certain Molecular Species of Liver Microsomal (A, B) and Plasma (C, D) Phosphatidylcholine in Rats Fed Different Levels of Corn Oil (1–20%).

Values are means±SEM for 6 rats. Values not sharing the same letters are significantly different at $p<0.05$.

○, rats fed control diets; ●, rats fed *L. edodes*-supplemented diets.

proportion in PC of linoleic acid or linoleic-acid-containing specific molecular species such as 16:0–18:2. Likewise, it appears that suppression of linoleic acid metabolism is more effective in decreasing arachidonic acid or arachidonic-acid-containing specific molecular species such as 18:0–20:4 in PC than decreased ingestion of linoleic acid. These phenomena might be accounted for by ingested linoleic acid being readily metabolized into arachidonic acid in rats, with their high $\Delta 6$-desaturase activity.[14]

The mechanisms of the hypocholesterolemic action of *L. edodes* and eritadenine have not yet been fully elucidated, but changes caused by eritadenine in phospholipid and fatty acid profiles may be involved.[5,8] In rats fed cholesterol-free diets, the major plasma lipoprotein is HDL rather than LDL or VLDL. The uptake of cholesteryl ester of reconstituted HDL by the liver is stimulated most by 16:0–18:2 when various PC molecular species are used in reconstitution of HDL particles,[15] suggesting that treatment to increase 16:0–18:2 PC molecular species of plasma HDL may decrease the plasma cholesterol concentration. In support of this, the proportion of 16:0–18:2 molecular species in plasma PC was much increased by dietary *L. edodes*, and the effect of the mushroom was greater than that of dietary corn oil. There was significant negative correlation between the plasma total cholesterol concentration and the proportion of 16:0–18:2 molecular species in plasma PC ($r=-0.90$, $p<0.001$). The hypocholesterolemic effect of dietary linoleic acid is smaller in rats fed a cholesterol-free diet than in rats fed a cholesterol-enriched diet. In our study also, in which diets were cholesterol-free, the hypocholesterolemic effect of dietary corn oil was negligible. This findings was consistent with the increase in the proportion of 16:0–18:2 molecular species in plasma PC caused by dietary corn oil being smaller than that caused by *L. edodes*. In conclusion, it is likely that suppression of linoleic acid metabolism, rather than increased ingestion of linoleic acid, is an effective way to increase the proportion of linoleic acid and linoleic-acid-containing specific molecular species in PC and to lower plasma cholesterol in rats fed a cholesterol-free diet.

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