JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Profiling of Hepatic Gene Expression of Mice Fed with Edible Japanese Mushrooms by DNA Microarray Analysis: Comparison among *Pleurotus ostreatus*, *Grifola frondosa*, and *Hypsizigus marmoreus*

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ABSTRACT: To compare and estimate the effects of dietary intake of three kinds of mushrooms (*Pleurotus ostreatus*, *Grifola frondosa*, and *Hypsizigus marmoreus*), mice were fed a diet containing 10−14% of each mushroom for 4 weeks. Triacylglycerol in the liver and plasma decreased and plasma cholesterol increased in the *P. ostreatus*-fed group compared with those in the control group. Cholesterol in the liver was lower in the *G. frondosa*-fed group than in the control group, but no changes were found in the *H. marmoreus*-fed group. DNA microarray analysis of the liver revealed differences of gene expression patterns among mushrooms. *Ctp1a* and *Fabp* families were upregulated in the *P. ostreatus*-fed group, which were considered to promote lipid transport and β-oxidation. In the *G. frondosa*-fed group, not only the gene involved in signal transduction of innate immunity via TLR3 and interferon but also virus resistance genes, such as *Mx1*, *Rsad2*, and *Oas1*, were upregulated.

KEYWORDS: food functionality, DNA microarray, *Pleurotus ostreatus*, *Grifola frondosa*, *Hypsizigus marmoreus*, lipid metabolic process, *Mx1*

INTRODUCTION

Recently, the number of people with lifestyle-related diseases such as hypertension, diabetes, or allergy has increased. Lifestyle-related diseases and maintenance and enhancement of the immune system are closely related to eating habits; therefore, functionality of foods attracts attention for the prevention and improvement of symptoms.

Mushrooms have been considered to have physiological effects on the maintenance and improvement of health for a long time. Extensive studies have demonstrated that mushrooms showed effects of improvement of lipid metabolism and modulation of the immune system, such as antidiabetic effects,[1−3] cholesterol-lowering effects,[4−7] antiatherogenic effects,[8] antihypertensive effects,[9] inhibitory effects on inflammatory bowel disease,[10] antiallergic effects,[11] and maintenance[12,13] and enhancement[14−16] of the immune system. The main component of the cell wall of mushrooms, β-glucan, has been shown to have effects of improvement of lipid metabolism[17] and enhancement of the immune system.[13−16] Besides β-glucan, the effects of heteropolysaccharide[18] and the low-molecular-weight protein fraction[19] of *Grifola frondosa* on the immune system have been reported. For evaluation of the function of food, elucidation of *in vivo* evidence and the mechanisms involved is needed. However, there are few reports on the elucidation of these physiological effects of mushrooms and their mechanism based on gene expression. As mentioned above, mushrooms have various physiological effects such as health maintenance and/or prevention of diseases; therefore, it is considered that mushroom dietary intake affects various genes *in vivo*.

DNA microarray technology is a technique for comprehensive gene expression analysis and has been used in various fields, such as basic biology, medical science, and agriculture. DNA microarray analysis of gene expression by food intake has enabled us to reveal the fluctuation of various metabolisms and signal transmission comprehensively. For this reason, DNA microarray has been used to evaluate the function of food and determine the mechanism involved;[20−23] by this approach, discovery of novel beneficial effects of food can be expected.

In this study, to clarify the differences of the dietary function of *Pleurotus ostreatus*, *G. frondosa*, and *Hypsizigus marmoreus*, which are mushrooms routinely eaten in Japan, normal mice were fed a diet containing each of these mushrooms for 4 weeks, and the hepatic gene expression patterns were analyzed using DNA microarray.

MATERIALS AND METHODS

Experimental Samples. The mushrooms used in this study were *Pleurotus ostreatus* (strain: Po89-1), *Grifola frondosa* (strain: Gf433), and *Hypsizigus marmoreus* (strain: Hm219). They were cultured at Hokkaido Research Organization Forest Products Research Institute. Basic medium was composed of larch (*Larix kaempferi*) sawdust for *P. ostreatus*, and birch (*Betula ermanii* Cham.) sawdust for *G. frondosa* and *H. marmoreus*.[24−26] The harvested fruit-bodies were autoclaved at 100 °C for 10 min, freeze-dried, and powdered.

Animals and Diets. The experimental diet was prepared according to the AIN-76 formulation.[27] Dietary fiber content in mushroom was calculated according to *Standard Tables of Food Composition in Japan*,[28]

Received: June 28, 2011
Accepted: August 30, 2011
Revised: August 30, 2011
Published: September 12, 2011

Table 1. Composition of the Diets (%)

component	dietary group			
	control	*P. ostreatus*	*G. frondosa*	*H. marmoreus*
sucrose	50.0	43.6	47.2	46.1
casein	20.0	18.9	18.9	19.1
corn starch	15.0	14.2	14.2	14.3
corn oil	5.0	4.7	4.7	4.8
cellulose	5.0	0.0	0.0	0.0
AIN-76 mineral mixture	3.5	3.3	3.3	3.3
AIN-76 vitamin mixture	1.0	0.9	0.9	1.0
DL-methionine	0.3	0.3	0.3	0.3
choline bitartrate	0.2	0.2	0.2	0.2
mushroom powder	0.0	13.9	10.4	10.9
total	100.0	100.0	100.0	100.0

and the cellulose powder in the control diet was substituted by mushroom powder in the experimental diet. The contents of *P. ostreatus*, *G. frondosa*, and *H. marmoreus* powder in the experimental diet were 13.9%, 10.4%, and 10.9%, respectively. At the same time, the composition of sucrose was modified (Table 1).

Four-week-old male ICR mice were obtained from CLEA Japan Inc. (Tokyo). The mice were given free access to AIN-76 pellets for 1 week for adaptation. They were randomly divided into 4 groups of 5 animals each and fed the experimental diets shown in Table 1 for 4 weeks. Mice were kept in an air-conditioned room at a temperature of 23 ± 2 °C with 40 ± 10% relative humidity and a 12 h light/dark cycle and given free access to experimental diet and water. Mice were fasted for 16 h before being anesthetized with diethyl ether. Blood was collected from the hearts, and the livers were isolated immediately. The livers were stored at −80 °C in RNA*later* (Ambion, Austin, TX) until RNA extraction. All mice were treated according to the guidelines for experimental animals of Obihiro University of Agriculture and Veterinary Medicine, Japan.

Analysis of Plasma and Liver Lipids. The blood was separated into plasma by centrifugation (4 °C, 3500 rpm, 15 min). The concentrations of plasma total cholesterol, HDL-cholesterol, triacylglycerol (TG), and phospholipid were measured using commercial enzymatic test kits (cholesterol E-test, HDL-cholesterol E-test, triacylglycerol E-test, phospholipid C-test Wakos, respectively; Wako Pure Chemical Industries, Osaka, Japan). Liver lipids were extracted according to the method of Danno et al.,[29] and the total cholesterol, TG, and phospholipid in the extracts were measured using the kits. Statistical analyses were performed by ANOVA with Dunnett's multiple comparison test ($P < 0.05$) using JMP 5.0.1 J (SAS Institute, Cary, NC).

DNA Microarray Analysis. Total RNA was isolated from the livers using SV total RNA isolation system (Promega, Madison, WI), and its quality was checked by its absorbance at 260 and 280 nm and by electrophoresis. Equal amounts of the RNA from five mice of each experiment group were pooled to normalize individual differences for DNA microarray analysis.

Labeled cRNA was synthesized using 2 μg of total RNA as the template with GeneChip one-cycle target labeling and control reagents (Affymetrix, Santa Clara, CA). The cRNA was fragmented according to the standard protocol and then hybridized at 45 °C for 16 h to GeneChip mouse genome 430A 2.0 array (Affymetrix), which represents about 12,000 transcripts. The array was washed and stained using GeneChip Fluidics Station 450 and GeneChip hybridization, wash, and stain kit (Affymetrix), and then scanned using GeneChip Scanner 3000 (Affymetrix). The collected fluorescence signals were background-corrected and converted into numeric data with the GeneChip operating software (Affymetrix). Gene Spring GX 7.3.1 (Agilent Technologies, Santa Clara, CA) was used for normalization of these data and comparison of these expression levels. At first, we extracted the probes with data flag "presence". Next, we identified the probes for which the signal level was up- or downregulated more than 1.5-fold compared with that of the control group. Functional clustering for the differentially expressed genes was assigned according to the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 (http://david.abcc.ncifcrf.gov/, Huang et al. 2009) at "medium" stringency based on the Gene Ontology (GO) biological process. Moreover, differentially expressed genes were categorized and mapped onto pathways using KEGG pathway database (http://www.kegg.jp/ja/, Kanehisa et al. 2000).

RESULTS AND DISCUSSION

Effect of Mushrooms on Liver and Plasma Lipids of Mice. Throughout the experimental period, no significant differences were found in the increase in body weights and the weights of epididymal fat among all groups (Table 2). Total cholesterol and TG contents in liver of the *P. ostreatus*-fed group were lower than those of the control group. In addition, total and HDL-cholesterol concentrations in plasma of the *P. ostreatus*-fed group were a little higher than those of the control group, while TG concentration of this group was around 50% lower than that of the control group. The liver weight and total cholesterol content in liver of the *G. frondosa*-fed group decreased compared with those of the control group; however, the plasma lipids were not affected. In addition, the liver and plasma lipids were not affected by feeding on *H. marmoreus*. The liver weights of the mushroom-fed groups decreased compared with that of the control group; however, gene expression levels of *Gpt2*, which encodes glutamic pyruvate transaminase (alanine aminotransferase) 2,[30] were not changed compared with that in the control group according to microarray data (data not shown). Therefore, it is considered that these mushrooms did not induce hepatotoxicity.

Hepatic Genes Whose Expression Levels Were Altered by Mushroom Intake. Microarray data with a "present flag" of each group were extracted. Numbers of genes whose expression levels were altered more than 1.5-fold compared with those of the control group are shown in Figure 1. Genes upregulated more than 1.5-fold compared with those of the control group numbered 946 in the *P. ostreatus*-fed group, 1102 in the *G. frondosa*-fed group, and 963 in the *H. marmoreus*-fed group. On the other hand, genes downregulated to less than 0.66-fold compared with those of the control group numbered 814 in the *P. ostreatus*-fed group, 1072 in the *G. frondosa*-fed group, and 682 in the *H. marmoreus*-fed group. More genes whose expression levels were altered were in the *G. frondosa*-fed group than in the other groups. There were 106 genes with altered expressions in common for the three kinds of mushrooms. In each of the groups of upregulated genes and downregulated genes, the number of genes with altered expressions in common for *G. frondosa* and *H. marmoreus* was more than 200, and the total in common for them was 488; therefore, it was supposed that these two mushrooms have an active factor in common.

Gene Expression Analysis of the Effects of Mushrooms on Plasma and Liver Lipids. As mentioned above, total cholesterol content in liver decreased and the plasma cholesterol concentration increased in the *P. ostreatus*-fed group compared with those of the control group. In addition, TG levels of the group decreased in the liver and plasma (Table 2). To characterize the mechanism involved, we focused on the gene expression of lipid metabolism-related genes based on the microarray data. There

Table 2. Metabolic Parameters of Mice Fed Experimental Diets for 4 Weeks[a]

	control	*P. ostreatus*	*G. frondosa*	*H. marmoreus*
initial BW[b] (g)	33.9 ± 2.2	30.7 ± 1.2	33.1 ± 1.1	32.1 ± 1.3
final BW[c] (g)	39.0 ± 3.9	36.2 ± 1.3	36.6 ± 0.6	37.2 ± 2.0
epididymal fat (g)	1.1 ± 0.4	0.9 ± 0.3	0.7 ± 0.1	1.1 ± 0.2
liver				
liver weight (g)	2.0 ± 0.4	1.8 ± 0.2	1.4 ± 0.1*[d]	1.6 ± 0.2
total lipid content (%)	7.5 ± 2.4	5.7 ± 1.2	6.6 ± 1.2	9.1 ± 1.8
total cholesterol content (%)	0.53 ± 0.15	0.34 ± 0.03*	0.38 ± 0.02*	0.40 ± 0.04
triacylglycerol content (%)	4.2 ± 2.0	2.3 ± 1.3*	2.8 ± 1.0	5.5 ± 1.8
plasma lipid				
total cholesterol/PL	0.47 ± 0.05	0.63 ± 0.05*	0.44 ± 0.05	0.45 ± 0.03
HDL-cholesterol/PL	0.40 ± 0.06	0.48 ± 0.02*	0.39 ± 0.05	0.36 ± 0.03
triglyceride/PL	0.86 ± 0.27	0.45 ± 0.16*	0.76 ± 0.19	1.18 ± 0.25

[a] Values are means ± SD ($n = 5$). [b] Initial body weight was measured before starting dietary protocols. [c] Final body weight was measured before sacrifice. [d] Asterisks (*) indicate differences from the control group at $P < 0.05$ by Dunnett's multiple comparison test.

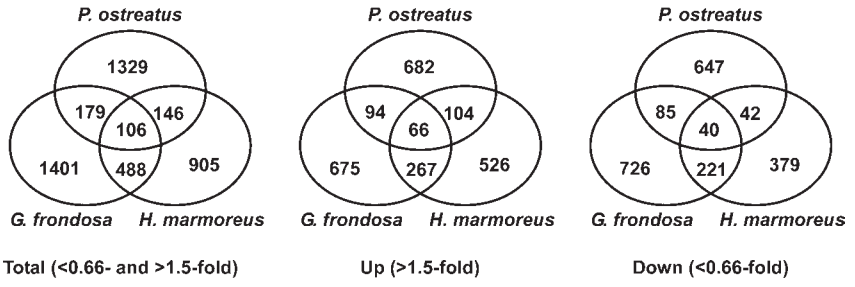

Figure 1. Venn diagram of the probes altered (<0.66- or >1.5-fold) by dietary intake of mushrooms.

were few lipid metabolism-related genes that were altered by *P. ostreatus* feeding. However, significant upregulation of *Cpt1a*, which encodes carnitine palmitoyltransferase, was observed in *P. ostreatus*-fed mice, and the level was 9.15-fold higher than that in the control mice (Table 3). *Cpt1a* is a key enzyme for the regulation of long-chain fatty acid β-oxidation in liver mitochondria.[31] By converting long-chain acyl-CoA into acylcarnitine, *Cpt1a* regulates the transport of cytosolic long-chain acyl-CoA into mitochondria, in which β-oxidation occurs. It is considered that the increase of the *Cpt1a* level promoted fatty acid oxidation and led to the decrease of TG in liver and plasma. On the other hand, although plasma total cholesterol increased in the *P. ostreatus*-fed group, among hepatic genes involved in cholesterol metabolism, the level of *Ldlr*, which encodes low-density-lipoprotein (LDL) receptor, was 1.96-fold that of the control group. It is supposed that cholesterol clearance from blood is accelerated through the LDL receptor.[32] As for cholesterol biosynthetic process-related genes, the levels of *Idi1* (isopentenyl-diphosphate Δ-isomerase, 4.72-fold), *Cyp51* (lanosterol 14-α-demethylase, 2.62-fold), and *Dhcr24* (24-dehydrocholesterol reductase, 2.04-fold) increased by *P. ostreatus* feeding (Figure 2), but total cholesterol in liver decreased significantly (Table 2). Hence, it is supposed that the expression levels of these enzyme genes did not markedly affect cholesterol synthesis in liver. Further studies on the factors involved in the increase in plasma cholesterol specific to *P. ostreatus* are needed.

Pck1, which encodes phosphoenolpyruvate carboxykinase, was upregulated more than 2-fold in the *G. frondosa*-fed group (Table 3). *Pck1* is a main control point for the regulation of gluconeogenesis. By hunger, insulin level decreases and glucagon level increases, and then phosphoenolpyruvate is synthesized from oxaloacetate by *Pck1* and provided for gluconeogenesis. On the other hand, downregulation of *G6pc* (0.55-fold), which encodes glucose-6-phosphatase and catalyzes glucose production, may control the rise of free glucose content toward glycogen synthesis and prevent the blood sugar level from rising too high. Thus, it is considered that use of a large amount of phosphoenolpyruvate for gluconeogenesis inhibited the pathway toward cholesterol synthesis from pyruvate via acetyl CoA in *G. frondosa*-fed mice. This probably caused the decrease of cholesterol level in liver.

Effects of *H. marmoreus* on the Expression of Lipid Metabolism-Related Genes. The plasma and liver lipid parameters were not affected by *H. marmoreus* feeding (Table 2). However, the gene expression of lipid metabolism-related genes showed significant changes in microarray analysis (Table 3). The genes that were expressed more than 1.5-fold in the *H. marmoreus*-fed group compared with those in the control group were classified into functional categories using DAVID. The top-ranked category was lipid metabolic process (GO:0006629) (Table 4). In this category, cholesterol (steroid) biosynthetic process (GO:0006695) and isoprenoid (terpenoid backbone) biosynthetic process (GO:0008299) were included. Furthermore, the genes categorized into the lipid metabolic process (GO:0006629) were classified according to metabolic pathways by KEGG (Table 5). Many upregulated genes were found in steroid biosynthesis, terpenoid backbone biosynthesis, PPAR signaling pathway, steroid hormone biosynthesis, androgen and estrogen metabolism, adipocytokine signaling pathway, and butanoate metabolism. In particular, the genes belonging to the former three pathways showed

Table 3. Expression Change (vs Control Group) of Hepatic Genes Involved in Lipid Metabolism by Mushroom Intake

			fold change			
GenBank accession	gene symbol	gene name	P^a	G^b	H^c	KEGG pathway
BB705380	*Hmgcs1*	3-hydroxy-3-methylglutaryl-coenzyme A synthase 1	1.35	1.09	1.52	T[d]
BB123978	*Hmgcr*	3-hydroxy-3-methylglutaryl-coenzyme A reductase	1.09	0.90	1.57	T
NM_138656	*Mvd*	mevalonate (diphospho) decarboxylase	—[e]	—	2.86	T
BC004801	*Idi1*	isopentenyl-diphosphate Δ-isomerase	4.72	0.97	5.41	T
BI247584	*Fdps*	farnesyl diphosphate synthetase	1.70	—	2.04	T
NM_010282	*Ggps1*	geranylgeranyl diphosphate synthase 1	1.70	1.61	2.58	T
AK014742	*Lss*	lanosterol synthase	1.62	1.45	2.27	S
NM_020010	*Cyp51*	cytochrome P450, family 51	2.62	1.10	2.26	S
BC014769	*Tm7sf2*	transmembrane 7 superfamily member 2	1.27	0.86	1.48	S
AK005441	*Sc4 mol*	sterol-C4-methyl oxidase-like	1.85	1.22	3.63	S
BC019945	*Nsdhl*	NAD(P) dependent steroid dehydrogenase-like	1.18	1.16	1.64	S
NM_010476	*Hsd17b7*	hydroxysteroid (17-β) dehydrogenase 7	1.37	1.19	1.49	S, Sh, An
BG295389	*Dhcr24*	24-dehydrocholesterol reductase	2.04	0.50	0.32	S
NM_007856	*Dhcr7*	7-dehydrocholesterol reductase	1.11	1.67	1.66	S
BC026757	*Hsd3b2*	hydroxy-Δ-5-steroid dehydrogenase, 3β- and steroid Δ-isomerase 2	1.19	0.61	1.87	Sh, An
BB825787	*Srd5a2l*	steroid 5α-reductase 2-like	1.19	1.14	0.78	Sh, An
AI891467	*Akr1d1*	aldo-keto reductase family 1, member D1	1.34	1.17	1.78	Sh, An, Pr
NM_053188	*Srd5a2*	steroid 5α-reductase 2	—	—	2.76	Sh, An
NM_007824	*Cyp7a1*	cytochrome P450, family 7, subfamily a, polypeptide 1	1.38	0.37	0.63	Sh, Pr, P
NM_018887	*Cyp39a1*	cytochrome P450, family 39, subfamily a, polypeptide 1	0.76	0.46	0.24	Pr
BB822856	*Pip5k1b*	phosphatidylinositol-4-phosphate 5-kinase, type 1 alpha	1.03	0.88	1.40	E
NM_008876	*Pld2*	phospholipase D2	1.29	0.66	1.75	E
AF425607	*Ldlr*	low density lipoprotein receptor	1.96	1.03	1.57	E
NM_008061	*G6pc*	glucose-6-phosphatase, catalytic	0.78	0.55	0.48	Ad
BB756794	*Prkag2*	protein kinase, AMP-activated, γ2 noncatalytic subunit	1.24	1.50	1.46	Ad
AF108215	*Prkab1*	protein kinase, AMP-activated, β 1 noncatalytic subunit	0.90	1.36	1.86	Ad
BB225177	*Pck1*	phosphoenolpyruvate carboxykinase 1	1.20	2.71	2.03	Ad, P
BB021753	*Cpt1a*	carnitine palmitoyltransferase 1a	9.15	1.02	1.88	Ad, P
AV236319	*Cpt2*	carnitine palmitoyltransferase 2	0.25	0.62	0.65	P
NM_010174	*Fabp3*	fatty acid binding protein 3, muscle and heart	—	1.27	1.72	P
BC002148	*Fabp4*	fatty acid binding protein 4, adipocyte	0.89	0.71	1.55	P
BC002008	*Fabp5*	fatty acid binding protein 5, epidermal	3.81	0.78	2.52	P
BC003305	*Lpl*	lipoprotein lipase	0.60	1.62	0.64	P

[a] *P. ostreatus.* [b] *G. frondosa.* [c] *H. marmoreus.* [d] T, terpenoid backbone biosynthesis; S, steroid biosynthesis; Sh, steroid hormone biosynthesis; An, androgen and estrogen metabolism; Pr, primary bile acid biosynthesis; E, endocytosis; Ad, adipocytokine signaling pathway; P, PPAR signaling pathway. [e] The probes with data flags "marginal" or "absent".

high expression levels. The expression levels of 33 genes belonging to these seven lipid pathways are shown in Table 3. In terpenoid backbone biosynthesis, *Hmgcs1*, synthesizing enzyme for HMGCoA from acetoacetyl-CoA, and *Hmgcr*, which encodes HMGCoA reductase and is involved in the next rate-determining step, were upregulated by 1.5-fold. Downstream, *Mvd* (mevalonate decarboxylase, 2.86-fold), *Idi1* (5.41-fold), *Fdps* (farnesyl diphosphate synthetase, 2.04-fold), and *Ggps1* (geranylgeranyl diphosphate synthase, 2.58-fold) were also upregulated (Figure 2). In the steroid biosynthesis pathway, which synthesizes sterols from farnesyl diphosphate and geranylgeranyl diphosphate synthesized in terpenoid backbone biosynthesis, *Sqle* (squalene epoxidase, 4.76-fold), *Lss* (lanosterol synthase, 2.27-fold), *Cyp51* (2.26-fold), and *Sc4 mol* (sterol-C4-methyl oxidase, 3.63-fold) were all upregulated. According to these results, it is considered that cholesterol synthesis in the liver increased. On the other hand, in steroid hormone (C_{18}-, C_{19}-, and C_{21}-steroids) biosynthesis, which synthesizes steroid hormones from cholesterol (Figure 2), *Star*, which converts cholesterol to pregnenolone, was upregulated by 10.59-fold. In addition, *Hsd3b2*, which encodes hydroxy-Δ-5-steroid dehydrogenase, 3β- and steroid Δ-isomerase 2, was upregulated. By contrast, upregulated genes in primary bile acid biosynthesis, which involves synthesis of bile acids from cholesterol, were not found, and gene expression levels of ABC transporter involved in cholesterol efflux were also not changed (data not shown). It is known that dietary fiber reduces the adsorption of cholesterol. Dietary fiber content of *H. marmoreus* is similar to that of the two other kinds of mushrooms. In conclusion, cholesterol synthesis was promoted, but the cholesterol was rapidly converted to steroid hormone, for example, progesterone; therefore, cholesterol content might not increase by *H. marmoreus* feeding. In addition, upregulation of *Ldlr* (1.57-fold) (Table 3) indicated the possibility of enhancement of the uptake of cholesterol from blood to liver. Consequently, the cholesterol

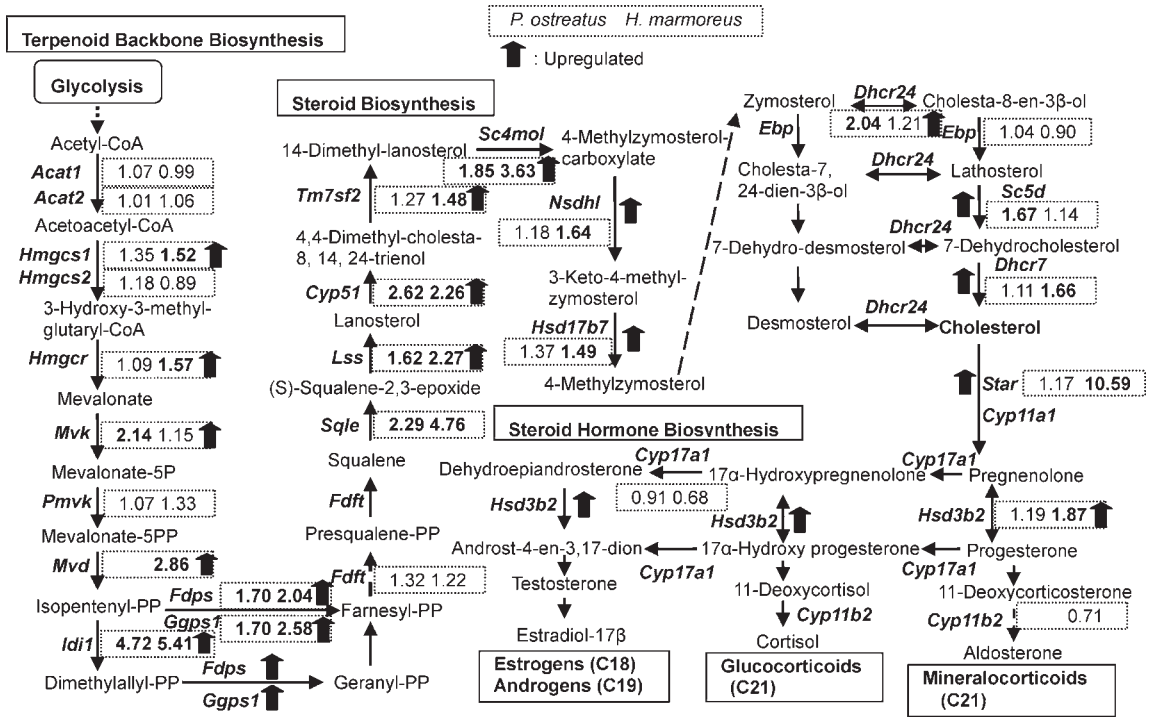

Figure 2. Gene expression level in steroid biosynthetic pathway induced by dietary intake of *P. ostreatus* and *H. marmoreus*.

Table 4. Functional Annotation Clustering of Genes Whose Expression Levels Were Altered by Mushroom Intake

GO number	GO term (biological process)	number of upregulated genes[a]		
		P. ostreatus	*G. frondosa*	*H. marmoreus*
GO:0006629	Lipid metabolic process			59
GO:0006695	...Cholesterol biosynthetic process			12
GO:0008299	...Isoprenoid biosynthetic process			6
GO:0009615	Response to virus		14	
GO:0002252	...Immune effector process		21	
GO:0002460	...Adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains		12	
GO:0019724	...B cell-mediated immunity		10	
GO:0016064	...Immunoglobulin-mediated immune response		9	
GO:0002376	Immune system process			55
GO:0042110	...T cell activation			15
GO:0042113	...B cell activation			9

[a] The number of genes upregulated by mushroom belonging to the GO category ($P < 0.05$).

taken to the liver is also considered to be metabolized to steroid hormones. In our study, steroid hormone concentration was not assayed; therefore, further investigation is needed to clarify what kind of steroid hormone would actually fluctuate in terms of its expression level.

Among the hepatic genes involved in PPAR signaling pathway or adipocytokine signaling pathway, the expression levels of *Cpt1a* (2.08-fold), fatty acid binding proteins *Fabp3* (1.72-fold), *Fabp4* (1.55-fold), and *Fabp5* (2.52-fold), and *Pck1*, 2.03-fold were high in *H. marmoreus*-fed mice (Table 3; Figure 3). These results suggested that the fatty acid transport was activated and the fatty acids were transported to mitochondria in the liver of *H. marmoreus*-fed mice. However, the expression level of *Cpt1a* in the group was one-fifth of that in the *P. ostreatus*-fed group, so TG was thought not to decrease. Suppressive effects of *H. marmoreus* on cholesterol have been reported.[7,8] In the present study, the suppressive effect was not observed. This difference may be because high-fat diets were used in these reported studies, but we used normal-fat diets in this study.

Effects of Mushrooms on the Expression of Immune System-Related Genes. By the functional analysis according to the GO biological process, *G. frondosa* was found to affect the expression of immune response-related genes significantly (Table 4). In upregulated genes (>1.5-fold) of the *G. frondosa*-fed group,

Table 5. Numbers of Genes Whose Expression Levels Were Altered (<0.66- or >1.5-fold) by *H. marmoreus* Intake

path code	function	number of altered genes: up	number of altered genes: down	*P* value
mmu00100	steroid biosynthesis	9	1	2.17×10^{-10}
mmu00900	terpenoid backbone biosynthesis	7	0	2.32×10^{-7}
mmu03320	PPAR signaling pathway	7	5	1.92×10^{-5}
mmu00140	steroid hormone biosynthesis	4	3	1.10×10^{-4}
mmu00150	androgen and estrogen metabolism	4	1	4.00×10^{-4}
mmu04920	adipocytokine signaling pathway	5	1	5.70×10^{-3}
mmu00650	butanoate metabolism	5	0	7.19×10^{-3}
mmu00600	sphingolipid metabolism	1	3	1.02×10^{-2}
mmu00120	primary bile acid biosynthesis	1	3	1.10×10^{-2}

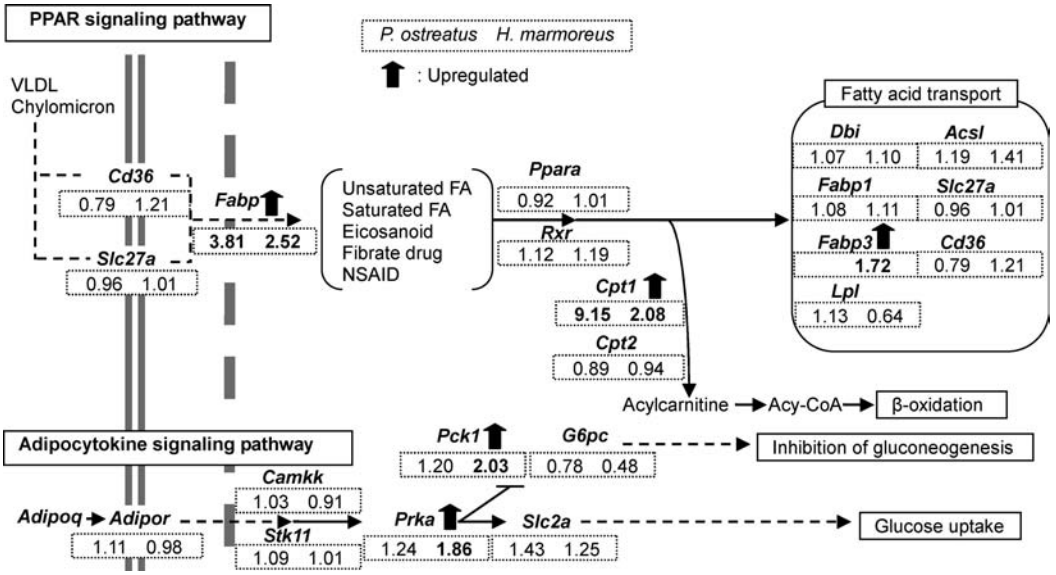

Figure 3. Gene expression level in PPAR signaling pathway induced by dietary intake of *P. ostreatus* and *H. marmoreus*.

response to virus (GO:0009615) ranked in the top cluster. In addition, B cell-mediated immunity (GO:0019724) and immunoglobulin-mediated immune response (GO:0016064) were also ranked highly; therefore, it is supposed that *G. frondosa* may modulate the immune system.

The liver, which is well-known as an organ controlling metabolism, detoxification, and storage of various compounds, is also important as an immune organ. NK cells, macrophages, and Kupffer cells are immune cells that exist in liver at high levels. As a result of DNA microarray analysis of hepatic genes, genes involved in the response to virus whose expressions were altered (<0.66- or >1.5-fold) by *G. frondosa* feeding compared with those of the control group were found to number 18. Eight of them were upregulated more than 2-fold compared with that in the control group (Table 6). In particular, the levels of *Mx1* (myxovirus resistance 1) and *Rsad2* (radical *S*-adenosyl methionine domain containing 2) were significantly high, 3.73- and 4.14-fold, respectively. *Mx1* is an influenza virus resistance gene.[33,34] *Rsad2* is an HIV and hepatitis C virus resistance gene.[35,36] Similarly, *Oas1* (2′,5′-oligoadenylate synthetase 1, 2.07-fold), known as a virus resistance gene, was also upregulated.[37] *Mx1*, *Rsad2*, and *Oas1* are interferon-regulated virus resistance genes.[38,39] Moreover, the expression levels of other interferon-regulated virus resistance genes, *G1p2* (interferon, α-inducible protein, 2.36-fold), *Ifi27* (interferon, α-inducible protein 27, 2.42-fold), and *Irf7* (interferon regulatory factor 7, 3.46-fold), increased significantly.[33] Masuda et al. reported that polysaccharide extracts from *G. frondosa* enhanced interferon γ.[18] In the present study, genes of interferon and interferon receptor showed little or no change in expression by *G. frondosa* intake (data not shown), but it is supposed that interferon signal transduction was enhanced according to the gene expression profiling. In addition, the expression level of *Tlr3* (Toll-like receptor 3), which acts on signal transduction of innate immunity, was upregulated 2.48-fold compared with that of the control group (Figure 4). Downstream, *Ikbke* (inhibitor of kappaB kinase ε) and *Irak4* (interleukin-1 receptor-associated kinase 4) were upregulated by 1.65-fold and 1.96-fold, respectively. TLR3 is a particularly important receptor in the signal transduction pathway for recognition of influenza virus. Therefore, it is supposed that *G. frondosa* dietary intake enhanced the protection against infection by influenza virus among others by increase in interferon via TLR3, induction of virus resistance genes, and activation of NK cells, as well as other factors.

Table 6. Expression Change (vs. Control Group) of Hepatic Genes Involved in the Response to Virus by Mushroom Intake

			fold change		
GenBank accession	gene symbol	gene name	P^a	G^b	H^c
BC018470	*Oas1a*	2′,5′-oligoadenylate synthetase 1A	0.92	2.07	–[d]
AK019325	*G1p2*	interferon, α-inducible protein	0.77	2.36	–
NM_011579	*Tgtp*	T-cell specific GTPase 2	1.30	2.50	1.10
BG095529	*Becn1*	beclin 1, autophagy related	0.48	0.65	0.79
BE911144	*Eif2ak2*	eukaryotic translation initiation factor 2-α kinase 2	1.24	0.75	1.33
M29881	*LOC386462*	similar to MHC Q8/9d surface antigen	1.40	1.56	1.44
BC010602	*H2-Q1*	histocompatibility 2, Q region locus 1	2.26	1.73	1.45
BF148012	*Samhd1*	SAM domain and HD domain, 1	0.78	1.56	–
NM_016850	*Irf7*	interferon regulatory factor 7	0.84	3.46	1.75
AY090098	*Ifi27*	interferon, α-inducible protein 27	0.85	2.42	1.33
X14607	*Lcn2*	lipocalin 2	0.44	1.60	0.94
M21039	*Mx1*	myxovirus (influenza virus) resistance 1	0.32	3.73	–
BF319989	*Plscr1*	phospholipid scramblase 1	2.02	1.86	1.42
AV169424	*Polr3k*	polymerase (RNA) III (DNA directed) polypeptide K	2.08	1.63	0.69
BB741897	*Rsad2*	radical *S*-adenosyl methionine domain containing 2	1.25	4.14	1.37
NM_126166	*Tlr3*	toll-like receptor 3	1.93	2.48	1.20
NM_133212	*Tlr8*	toll-like receptor 8	–	0.31	0.59
AA215024	*Xpr1*	xenotropic and polytropic retrovirus receptor 1	0.85	1.99	1.05

[a] *P. ostreatus.* [b] *G. frondosa.* [c] *H. marmoreus.* [d] The probes with data flags "marginal" or "absent".

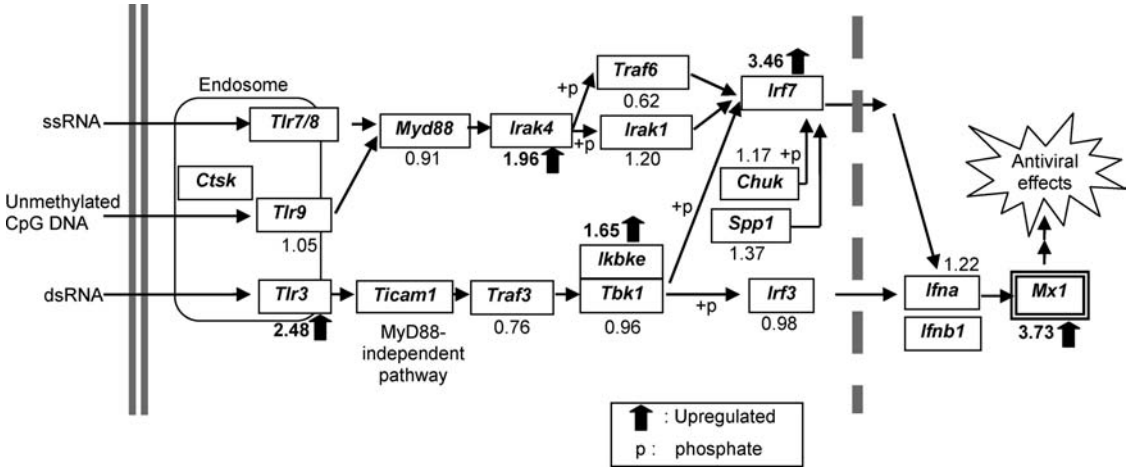

Figure 4. Gene expression level involved in the response to virus induced by dietary intake of *Grifola frondosa*.

In the *G. frondosa*-fed group, genes involved in activation of immune functions through acquired immunity by B cells (immunoglobulin-mediated immune response) were upregulated (Table 4). In this category, the gene expression level of *H2-Ea* (histocompatibility 2, class II antigen Eα) was increased 8.27-fold, and those of *H2-Ab1* (histocompatibility 2, class II antigen A, β1) and *H2-Q1* (histocompatibility 2, Q region locus 1), which encode components of the MHC class II antigen processing and presentation pathway, were increased more than 1.5-fold (data not shown).[40] *Icosl* (icos ligand), which is the ligand of *Icos* (inducible costimulator) and is needed to enhance T cell proliferation in humoral immune response,[41] was upregulated by 3.24-fold. In addition, *Igh-VJ558* (immunoglobulin heavy chain, J558 family) and *Igh-V3609N* (immunoglobulin heavy chain, V3609N nonproductive) were upregulated by 1.94- and 2.20-fold, respectively. Obi et al. reported that stimulation with *G. frondosa* extract on macrophages induced TNF-α production and had an inhibitory effect on the growth of influenza virus *in vitro*.[42] In the present study, the increase in TNF-α was not found by microarray data, but it was possible that *G. frondosa* activated the protection system against virus through not only the pathway via TLR3 but also antibody production of B cells.

Compared with the other mushroom-fed group, with regard to genes involved in the response to virus, genes that were upregulated more than 1.5-fold compared with their levels in the control group numbered four in the *P. ostreatus*-fed group and one in the *H. marmoreus*-fed group (Table 6). Thus, the expression levels of many genes involved in the response to virus were altered characteristically by *G. frondosa* intake.

In the *H. marmoreus*-fed group, expression changes were found in genes involved in immune pathways, for example, T cell activation (GO:0042110) and B cell activation (GO:0042113) (Table 4); therefore, it is supposed that *H. marmoreus* affected the immune system similarly to *G. frondosa*. Because the affected functional categories of *H. marmoreus* were different from those of *G. frondosa*, it is considered that the targets of the immune system were different between the two mushrooms. In the *P. ostreatus*-fed group, GO factors involved in the immune system were not significantly enriched compared with the entire data set (data not shown).

The main objective of this study is to examine the comprehensive changes in hepatic gene expression caused by ingestion of three kinds of mushrooms, to compare and estimate the effects of dietary intake of these mushrooms. Therefore, we did not explore the identification of any effective compounds contained in these mushrooms that affected gene expression. On the basis of previous studies,[13−17] it is considered that the possible effective compounds of these mushrooms are β-glucans. It has been reported that the glucan from *G. frondos*a with food functionality (grifolan) is composed of a β-(1,3)-D-glucan with one β-(1,6)-D-glucose side chain and the degree of branching is 1/3,[43] but the structure of β-glucan is different among different kinds of mushrooms,[43] and this variation may influence the differences in gene expressions of lipid metabolism and the immune system. Accordingly, further investigation is needed to determine the active components of these mushrooms. In addition, further examination is needed to elucidate the mechanism of physiological effects of the mushrooms.

In this study, on the basis of comprehensive gene expression profiling, *G. frondosa* is expected to prevent influenza virus infection, and *H. marmoreus* and *P. ostreatus* are expected to promote fat burning (β-oxidation). These results suggested that these mushrooms are valuable for prevention of infection by the enhancement of innate immunity or for improvement of lifestyle-related diseases such as obesity.

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ABBREVIATIONS USED

Cpt1a, carnitine palmitoyltransferase 1a; *Fabp*, fatty acid binding protein; TLR3, toll-like receptor 3; *Mx1*, myxovirus (influenza virus) resistance 1; *Rsad2*, radical S-adenosyl methionine domain containing 2; *Oas1*, 2′,5′-oligoadenylate synthetase 1; PPAR, peroxisome proliferator activated receptor; HMGCoA, hydroxymethylglutaryl-CoA; *Star*, steroidogenic acute regulatory protein; ABC, ATP-binding cassette

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