Japanese mushroom consumption alters the lipid metabolomic profile of high-fat diet-fed mice

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ABSTRACT

Mushrooms are familiar ingredients in Japanese cuisine and large numbers are consumed in Japan. Recently, we reported that the consumption of Japanese mushrooms suppressed the accumulation of visceral fat. The purpose of this study was to examine the alteration of lipid metabolism by Japanese mushrooms consumption in high-fat diet (HFD) mice. Multivariate analysis of serum, liver, adipose tissue, cecal contents, large intestinal and fecal lipids showed differing compositions in the mice that had consumed HFD or HFD supplemented with 3% freeze-dried mushroom mixture (HFMD). There were higher concentrations of diacylglycerol in the adipose tissue, non-esterified fatty acids in the serum, and triacylglycerol in the feces of the HFMD group. These results suggest that mushroom consumption promotes the degradation of lipids in visceral fat and limits the absorption of food lipids. Moreover, the HFMD group demonstrated higher concentrations of phospholipids, some of which contained odd-chain fatty acids. Thus, we speculated that the alteration of lipid metabolism in mice such that mushroom consumption prevent obesity progression, as demonstrated by metabolomic analysis.

1. Introduction

Obesity is a risk factor for many diseases, including cardiovascular disease, type 2 diabetes, cancer, and depression (Dandona et al., 2005; Faith et al., 2002; Hursting et al., 2012). The numbers of obese men and women have increased rapidly worldwide since the 1970s, and many researchers have investigated means of resolving this risk factor (NCD-Risk, 2016). To prevent or reduce obesity, the imbalance between energy intake and expenditure must be ameliorated using dietary and/or lifestyle interventions (Hofbauer, 2002). Japan is known to have fewer obese people than Western countries, and Japanese cuisine is considered to be one of the explanations for this difference (Iwagaki et al., 2017; Tsuduki et al., 2008). A distinctive feature of Japanese cuisine is the predominant use of ingredients such as vegetables, fish, soy beans, and mushrooms. In particular, various types of mushrooms are often used in Japanese cuisine, and about 16 g of mushrooms are consumed per person each day in Japan ("National Health and Nutrition Survey Report, 2017"). Mushrooms contain many useful nutrients, such as dietary fiber, vitamin B1, vitamin B2, vitamin B3 (niacin), vitamin B6, vitamin B9 (folic acid), and vitamin D (Valverde et al., 2015). Furthermore, they have been reported to have anti-obesity (Handayani et al., 2011; Iuchi et al., 2015; Mizutani et al., 2010; Yeh et al., 2014), immunomodulatory (Vetvicka and Vetvickova, 2014), anti-tumor (Masuda et al., 2013), anti-atherosclerotic (Mori et al., 2008), and anti-diabetic effects (Hong et al., 2007).

Our previous study showed that the consumption of a mixture of five types of Japanese mushrooms (Flammulina velutipes, Hypsizygus marmoreus, Lentinus edodes, Grifola frondosa, and Pleurotus eryngii) suppressed fat accumulation by inhibiting fatty acid synthesis and promoting lipolysis in the perinephric adipose tissue of mice (Shimizu et al., 2018). The weight of perinephric adipose tissue is significantly reduced during starvation compared to other adipose tissues (Hegarty and Kim, 1981; Suzuki and Koyanagi, 1968). Therefore, we hypothesized that perinephric adipose tissue might be more affected than other adipose tissue due to the inhibition of lipid absorption by mushrooms. In addition, it caused the proliferation of short-chain fatty acid (SCFA)- and some lactic acid-producing bacteria, possibly because of the dietary fiber content of the mushrooms. Moreover, this mushroom consumption increased the activity of hormone-sensitive lipase (HSL), which regulates lipolysis in adipocytes, and suppressed the expression of sterol regulatory element-binding factor 1 c (SREBP-1c), which regulates fatty acid synthesis, in the perinephric adipose tissue (Shimizu et al., 2018). These
findings are consistent with mushroom consumption suppressing visceral fat accumulation. However, whereas many studies have reported anti-obesity effects of mushrooms, few studies have analyzed the effects of the consumption of a mixture of mushrooms on obesity-related metabolism.

Recently, methods have been established for the comprehensive metabolomic analysis of blood, cells, tissues, feces, and food, which have permitted the elucidation of the effects of numerous interventions on metabolism (Beckonert et al., 2007; Wishart, 2008). Furthermore, it is likely that the metabolomic analysis of lipid species will lead to the identification of markers of pathologic features of lifestyle-related diseases that involve abnormalities of lipid metabolism, such as obesity and type 2 diabetes (Nam, Choi, Jung, Jung, Choi, Ryu, et al., 2015; Zhao et al., 2013). Lipids are major components of biologic membranes and play important functional roles, for example as energy sources and in signal transduction. Differences in the diet are reflected in differences in lipid metabolite concentrations (M. J. Kim, Yang, Kim, Ahn, Lee, Kim, et al., 2013; Nam, Choi, Choi, Kim, Kim, Jung, et al., 2018); for example, standard and high-fat diet consumption are associated with significant differences in the concentrations of lipid metabolites in the serum and liver (H.-J. Kim, Kim et al., 2010).

Our previous study showed that mushroom consumption is an effective strategy for the prevention of obesity (Shimizu et al., 2018). In the present study, we compared the serum, liver, adipose tissue, cecal, large intestinal, and fecal lipid profiles of mice consuming a normal diet, a high-fat diet, or a high-fat diet supplemented with the same mixture of mushrooms, to examine the effect of mushroom consumption on lipid metabolism in initial stage of obesity.

2. Materials and methods

2.1. Preparation of the mushroom mixture

Preparation of the mushroom mixture and its nutritional composition were described in our previous study (Shimizu et al., 2018). We used five types of mushrooms (F. velutipes, H. marmoreus, L. edodes, G. frondosa and P. eryngii) that consumed the most in Japan (“Survey on production forest products production statistics 2014”). F. velutipes was obtained from Mashgarden Corp. (Miyagi, Japan), and H. marmoreus, L. edodes, G. frondosa and P. eryngii were obtained from Hokuto Corp. (Nagano, Japan). The fresh fruiting bodies of the mushrooms were boiled in water for 10 min, and then the hot water-treated mushrooms and the water were freeze-dried. The self-sufficiency rate of mushrooms is very high in Japan. For this reason, we hypothesized the Japanese mushroom consumption is approximately equal to the domestic production. The freeze-dried mushroom powders were mixed according to the proportions of Japanese mushrooms produced, according to the Ministry of Agriculture, Forestry, and Fisheries of Japan (“Survey on production forest products production statistics 2014”).

2.2. Experimental diets

The mushroom mixture was mixed with a high-fat diet (Western Diet-D12079B, Research Diet Inc.). The high-fat diet contains 21 g of lipid per 100 g. Three test diets were prepared as follows: a normal diet (control; 98121701, Research Diet Inc.); a high-fat diet; and the high-fat diet containing the mushroom mixture at 3% w/w (ND, HFD, and HFMD groups, respectively). The intake of the test diet containing the
The mushroom mixture by the mice was calculated to be equivalent to a human intake of ~600 g of fresh mushrooms per day. To render the macronutrient composition and energy content of the HFD and HFMD equivalent, the contributions of the mushrooms were quantified and these amounts replicated in the HFD diet using an equivalent control mixture, comprising casein as the protein source, soy oil as the lipid source, corn starch as the carbohydrate source, pectin as the water-soluble dietary fibre source, and cellulose as the insoluble dietary fiber source, with the remaining mass being made up using cellulose (Shimizu et al., 2018). The final energy content per 100 g of each test diet was ND: 391 kcal, HFD: 460 kcal, and HFMD: 460 kcal.

### 2.3. Animals

All animal procedures were performed in accordance with the Animal Experimentation Guidelines of Tohoku University, and the animal protocol was approved by the Animal Use Committee at Tohoku University (Registration ID No. 2016AgA-009). Four-week-old male C57BL/6J mice (mean body mass: 18 g) were obtained from SLC, Inc. (Shizuoka, Japan). The mice were housed for the duration of the study under a 12 h/12 h light/dark cycle in a temperature and humidity-controlled room, and fed a standard rodent chow (CE-2, CLEA Japan, Inc., Tokyo, Japan) during a 1-week acclimation period. When 5 weeks old, the mice were randomly allocated to the three dietary groups (n = 8 per group; four mice per cage), and each group received ND, HFD, or HFMD and water ad libitum for 4 weeks. At the end of this 4-week period, blood samples were collected following decapitation under isoflurane anesthesia. Liver, perinephric adipose tissue, large intestine, and cecal contents were removed. Serum samples and organs were stored at −80 °C until use. Feces were collected after 4 weeks of test diet consumption, just after a routine cage change, pooled for each group, and stored at −80 °C until analyzed.

### 2.4. Serum, tissue, and fecal lipid extraction

Serum (10 μL), liver (60 mg), perinephric adipose tissue (10 mg), cecal contents (60 mg), large intestine (60 mg), and feces (60 mg) were transferred to 1.5 mL-tubes containing 3.0 mm zirconium beads and homogenized twice using a Micro Smash™ MS-100 (Tomy Digital Biology Co., Ltd., Tokyo, Japan) at 4,800 rpm in 1.0 mL methanol for 30 s. After homogenization, the mixtures were sonicated for 5 min and centrifuged for 1 min at 21,130 × g at 4 °C. The supernatants were filtered using a 0.2 μm PTFE filter (Agilent Technologies, California, USA) and transferred to vials (GL Sciences, Tokyo, Japan).

### 2.5. Conditions for the metabolomic analysis

Liquid chromatography (LC)-electrospray ionization (ESI)- mass spectrometry (MS)/MS analyses were carried out on a SCIEX X500R Q-
TOF mass spectrometer (Framingham, U.S.A.). Separations were performed on an iHILIC®-Fusion (100 × 2.1 mm) column (Hilicon, Umeå, Sweden). The column temperature was maintained at 40 °C and the autosampler temperature at 5 °C. Ten-microliter samples were injected. The binary gradient system included 10 mM ammonium formate in a water: acetonitrile mixture (95:5, v/v; solvent A) and 10 mM ammonium formate in a water: acetonitrile mixture (5:95, v/v; solvent B). The gradient profile was 95% A–5% B at 0–5 min, 5% A–95% B at 5–10 min, and 95% A–5% B at 10–15 min, and the flow rate was maintained at 0.2 ml/min. The mass spectrometer was operated in positive and negative ion modes, and data acquired in the mass range 50–1000 m/z. The total ion chromatogram was acquired using the following operation parameters: capillary voltages of +5,500 V and -4,500 V for the positive and negative modes, respectively, a nebulizer pressure of 413.8 kPa (60 psi), a drying gas pressure of 413.8 kPa (60 psi), a curtain gas pressure of 206.9 kPa (30 psi), a source temperature of 350 °C, a declustering potential of ±80 eV, and a collision energy of ±10 eV for single MS and ±55 eV for MS/MS.

### 2.6. Data processing

Alignment of the detected peaks was performed according to their m/z values and the retention times were normalized using MarkerView™ Software (version 1.2.1, AB SCIEX). Principal component analysis (PCA) Discriminant analysis (DA) results were processed using MarkerView™ Software. Assignment of the spectral peaks was performed using METLIN (https://metlin.scripps.edu) and the Human Metabolome Database (HMDB; http://www.hmdb.ca/). Heatmaps were generated using MultiExperiment Viewer Ver. 4.9.0 (Mev, http://www.tm4.org/mev/).
2.7. Biochemical analyses of serum and feces

Free fatty acid concentrations were measured using a NEFA C test kit (Wako Pure Chemical Industries, Osaka, Japan). The lipid compositions of feces were determined as described previously (Tsuyoshi Tsuduki, Yamamoto, Hatakeyama and Sakamoto, 2015). Triacylglycerol (TG) concentration was measured using a commercial enzymatic kit (Wako Pure Chemical, Osaka, Japan). The lipid compositions of the serum, liver, and adipose tissue in high-fat diet-fed mice showed that mushroom consumption increased the relative concentrations of lysophosphatidylcholine (LPC), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), sphingomyelin (SPM), and triacylglycerol (TG) (Figure 2a, Table 1). Analysis of these serum lipid concentrations that were significant when the clusters of the serum, liver, and adipose tissue in high-fat diet-fed mice. Heatmaps indicate the lipid metabolite composition of the samples among the ND, HFD, and HFMD groups. The results show that the clusters for each group separated in the positive and negative modes (Figure 1), suggesting that the body composition of the three groups differed as a result of the differing composition of their diets. There were particularly noticeable distances between the clusters of the HFD and HFMD groups in adipose tissue. Therefore, we speculated that mushroom consumption changed lipid metabolism in adipose tissue.

2.8. Statistical analysis

All statistical analyses were performed using KaleidaGraph (Hulinks Inc., Tokyo, Japan). Results are expressed as mean ± standard error (SE). Data were analyzed using one-way ANOVA, followed by the Tukey test. Differences was considered to be significant when \( p < 0.05 \).

3. Results and Discussion

3.1. Multivariate analysis of serum, liver, and adipose tissue after mushroom consumption

Lipid profiles of the serum, liver and adipose tissue in high-fat diet-fed mice can reflect the course of obesity development (Anjos et al., 2019; Nam et al., 2018). As shown in our previous study, there were no significant differences in body mass gain and energy intake among the groups. However the high-fat plus mushroom diet group showed significantly lower perinephric adipose tissue mass than the high-fat diet group (Shimizu et al., 2018). Here, serum, liver, and adipose tissue samples were pre-treated, and the compounds present were comprehensively analyzed using LC-QTOFMS. The analysts used both positive and negative modes, and identified 3,008 and 2,681 peaks in the serum, 2,970 and 2,555 peaks in the liver, and 2,959 and 2,506 peaks in the adipose tissue, respectively. All the peaks for each sample were analyzed by multivariate analysis (PCA-DA) to visualise the clusters and compare the composition of the samples among the ND, HFD, and HFMD groups. The results show that the clusters for each group separated in the positive and negative modes (Figure 1), suggesting that the body composition of the three groups differed as a result of the differing composition of their diets. There were particularly noticeable distances between the clusters of the HFD and HFMD groups in adipose tissue. Therefore, we speculated that mushroom consumption changed lipid metabolism in adipose tissue.

3.2. Effect of mushroom consumption on the lipid metabolite composition of serum, liver, and adipose tissue

We examined whether mushroom consumption altered the lipid profiles of high-fat diet-fed mice. Heatmaps indicate the lipid metabolite concentrations that were significantly higher or lower in the HFMD group than in the HFD group (Figure 2). Analysis of these serum lipid profiles showed that mushroom consumption increased the relative concentrations of lysophosphatidylcholine (LPC), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), sphingomyelin (SPM), and triacylglycerol (TG) (Figure 2a, Table 1). Analysis of the liver lipid profiles showed that mushroom consumption increased the relative concentrations of lipid metabolites.
| Proposed identity | Molecular formula | Ret. time | Precursor ions (m/z) | Difference (ppm) | Adduct | Relative intensity (ratio; ND = 1) | p-value (HD v.s. HDM) |
|------------------|-------------------|-----------|----------------------|------------------|--------|----------------------------------|-----------------------|
| LysoPC (16:0)    | C24H50NO7P        | 4.95      | 513.3782             | 23               | [M+NH4]+ | 1.00                              | 9.39                  | 14.88                | 0.000                |
| LysoPC (18:1)    | C26H52NO7P        | 6.99      | 522.3557             | 0                | [M+H]+   | 1.00                              | 9.01                  | 10.53                | 0.026                |
| LysoPC (22:6)    | C30H50NO7P        | 5.05      | 568.3405             | 1                | [M+H]+   | 1.00                              | 2.22                  | 2.86                 | 0.008                |
| PC (42:0)        | C50H100NO8P       | 7.04      | 872.7028             | 9                | [M-H]-   | 1.00                              | 0.92                  | 1.26                 | 0.005                |
| DG (28:0)        | C31H60O5          | 4.82      | 530.4808             | 5                | [M+NH4]+ | 1.00                              | 11.82                 | 18.79                | 0.000                |
| DG (28:1)        | C31H58O5          | 4.69      | 528.4636             | 2                | [M+NH4]+ | 1.00                              | 7.99                  | 12.54                | 0.000                |
| DG (30:0)        | C33H60O5          | 5.07      | 541.4814             | 2                | [M+H]+   | 1.00                              | 5.79                  | 8.33                 | 0.000                |
| DG (30:1)        | C33H58O5          | 4.96      | 539.4685             | 2                | [M+H]+   | 1.00                              | 7.28                  | 9.90                 | 0.005                |
| DG (30:2)        | C33H62O5          | 4.69      | 554.4778             | 0                | [M+NH4]+ | 1.00                              | 5.35                  | 7.74                 | 0.003                |
| DG (31:0)        | C34H64O5          | 4.81      | 555.4825             | 28               | [M+H]+   | 1.00                              | 7.61                  | 11.18                | 0.002                |
| DG (31:1)        | C34H62O5          | 4.99      | 570.5080             | 2                | [M+NH4]+ | 1.00                              | 3.37                  | 4.27                 | 0.017                |
| DG (31:2)        | C35H64O5          | 5.15      | 569.5128             | 2                | [M+H]+   | 1.00                              | 1.96                  | 2.68                 | 0.001                |
| DG (32:0)        | C35H62O5          | 5.44      | 587.4649             | 0                | [M+Na]+  | 1.00                              | 1.93                  | 2.54                 | 0.001                |
| DG (32:1)        | C35H60O5          | 5.12      | 559.4345             | 2                | [M+H]+   | 1.00                              | 5.73                  | 8.09                 | 0.000                |
| DG (33:2)        | C36H66O5          | 5.05      | 596.5249             | 0                | [M+NH4]+ | 1.00                              | 2.58                  | 3.33                 | 0.008                |
| DG (34:0)        | C39H74O5          | 5.48      | 640.5826             | 7                | [M+NH4]+ | 1.00                              | 1.90                  | 2.36                 | 0.014                |
| DG (34:5)        | C37H66O5          | 4.98      | 585.4299             | 38               | [M+H]-   | 1.00                              | 4.45                  | 7.45                 | 0.000                |
| DG (36:1)        | C41H68O5          | 5.41      | 641.5112             | 4                | [M+H]-   | 1.00                              | 1.74                  | 2.17                 | 0.015                |
| DG (36:2)        | C39H67D5O5        | 5.23      | 614.5064             | 24               | [M+H]-   | 1.00                              | 1.51                  | 2.46                 | 0.000                |
| DG (36:4)        | C39H68O5          | 5.2       | 613.5025             | 30               | [M+H]-   | 1.00                              | 1.55                  | 2.47                 | 0.000                |
| DG (36:5)        | C39H66O5          | 5.22      | 613.4837             | 27               | [M+H]-   | 1.00                              | 2.11                  | 3.06                 | 0.001                |
| DG (36:6)        | C39H64O5          | 5.01      | 611.4689             | 27               | [M+H]-   | 1.00                              | 0.73                  | 1.03                 | 0.013                |

Figure 3. Multivariate analysis of the cecal content, large intestinal, and fecal metabolites of mice fed ND, HFD, or HFMD. PCA-DA score plots obtained from LC-QTOF-MS spectra in positive and negative modes. ND: normal diet, HFD: high-fat diet, HFMD: high-fat diet plus mushroom mixture.
concentrations of fatty acid (FA), phosphatidic acid (PA), PC, phosphatidyl ethanolamine (PE), phosphatidyl glycerol (PG), PI, ceramide (Cer), glycosphingosaccharide (GSL), and diacylglycerol (DG). Furthermore, mushroom consumption increased the relative concentration of PS (44:8) in the liver, but reduced that of PS (40:4) (Figure 2b, Table 2). Finally, analysis of the adipose lipid profiles showed that mushroom consumption increased the relative concentrations of LPC, PC, and DG (Figure 2c, Table 3). Higher concentrations of LPC in the serum and adipose tissue have also been shown following green tea consumption, which also has an anti-obesity effect (Nam et al., 2018); therefore, LPC may participate in the suppression of fat accumulation by mushroom consumption. Next, we focused on the increase in the DG content of the adipose tissue. TG in adipose tissue is hydrolyzed by hormone-sensitive lipase (HSL), a lipolytic enzyme expressed in adipocytes, generating DG and non-esterified fatty acid (NEFA). NEFA is transported in the blood and used as an energy source in skeletal muscle and heart. To investigate the effect of mushroom consumption on lipolysis in adipose tissue, the concentration of NEFA was measured in the serum. This showed a tendency for NEFA concentration to be higher in the HFMD group than in the HFD group (p = 0.0971) (Figure 2d). In addition, mushroom consumption increased the relative concentrations of DG (p = 0.0335) and PG (p = 0.0171) in the adipose tissue (Figure 2c). These results imply that mushroom consumption promotes the degradation of TG in adipocytes. Furthermore, we have previously reported that mushroom consumption reduces white adipose tissue mass (Shimizu et al., 2018). Therefore, we speculate that the promotion of triglyceride hydrolysis is involved in the effect of mushroom consumption to suppress visceral fat accumulation.

In addition, DG is known to inhibit insulin signaling in liver and skeletal muscle (Erion and Shulman, 2010). However, there were no significant differences in the serum insulin concentration between the groups, and mushroom consumption tended to reduce serum glucose (Shimizu et al., 2018). Furthermore, adiponectin, which is known to ameliorate insulin resistance (Yamauchi et al., 2001), was present in higher concentrations in the HFMD group than the HFD group. Therefore, the increase in DG concentration in adipose tissue following mushroom consumption is not considered to induce insulin resistance.

### 3.3. Multivariate analysis of cecal, large intestinal, and fecal lipids after mushroom consumption

Subsequently, we determined the effects of mushroom consumption on the composition of the contents of the lower digestive tract. The collected cecal contents, large intestine, and feces were pre-treated, and the compounds contained in these samples were comprehensively analyzed using LC-QTOFMS. Analyses using both positive and negative modes identified 3,155 and 2,980 peaks in the cecal contents, 3,942 and 2,315 in the large intestine, and 3,271 and 2,175 in the feces, respectively. All the peaks detected for each sample were analyzed by
multivariate analysis (PCA-DA) to visualise the clusters and compare the lipid composition among the ND, HFD, and HFMD groups. The results show that the clusters for each group separated in the positive and negative modes (Figure 3), suggesting that the lower digestive tract contents differed in their lipid composition among the three groups, because of differences in the diet consumed. There were particularly noticeable distances among the cluster of three groups in feces.

3.4. Effects of mushroom consumption on the lipid profiles of cecal contents, large intestine, and feces

Heatmaps demonstrate the lipid metabolites that were present in significantly higher or lower concentrations in the HFMD group than the HFD group (Figure 4). Analysis of the lipid profiles of the cecal contents showed that mushroom consumption increased the relative concentrations of LPC, PC, and TG. There were no significant differences in the relative concentration of PE in the large intestine and feces. However, mushroom consumption increased the relative concentration of PE (36:5), and reduced that of PE (33:0) in the cecal contents. Mushroom consumption also reduced the relative concentration of MG in the cecal contents (Figure 4a, Table 4). Analysis of the lipid profiles of the large intestine showed that mushroom consumption increased the relative concentration of lysophosphatidic acid (LPA) (Figure 4b, Table 5). Analysis of the fecal lipid profiles showed that mushroom consumption reduced the relative concentration of FA (C18:2), but increased the relative concentrations of PC, DG, and TG (Figure 4c, Table 6).

We then focused on the increase in phospholipid content of the lower digestive tract. Dietary fiber alters the rate of intestinal cell turnover and viscous dietary fiber exfoliates intestinal cells (Jin et al., 1994; Tasman-Jones et al., 1982), which may aid in the prevention of infection and maintenance of barrier function in intestinal epithelial cells (Cliffe et al., 2005). PC is a major lipid component of cell membranes, and the mushroom-induced increase in PC in the lower digestive tract may be attributed to detached intestinal cells. A mushroom-induced increase in LPA was also identified in the large intestine, and LPA enhances intestinal epithelial wound healing by promoting intestinal epithelial cell

| Proposed identity | Molecular formula | Ret. time | Precursor ions (m/z) | Difference (ppm) | Aduct | Relative intensity (ratio; ND – 1) | p-value (HD v.s. HDM) |
|-------------------|-------------------|-----------|---------------------|-----------------|-------|-----------------------------------|----------------------|
|                   |                   |           | Observed            | Theoretical     |        | ND                                | HD                   | HDM                  |
| LysoPA (18:0)     | C21H45O6P         | 1.41      | 423.2776            | 424.2954        | [M-H] | 1.00                              | 0.97                 | 1.16                 | 0.010                |
| LysoPA (20:3)     | C23H41O17P        | 1.38      | 459.2445            | 460.259         | [M-H] | 1.00                              | 1.07                 | 1.43                 | 0.0064               |

Table 4. Lipid profiles of the cecal contents.
migration and proliferation (Sturm et al., 1999). Moreover, mushroom consumption tended to increase the weight and length of the large intestine (Shimizu et al., 2018). Taken together, these findings may suggest that mushroom consumption promotes intestinal turnover and has beneficial effects on maintains homeostasis intestinal epithelial.

Because mushroom consumption increased the fecal TG concentration, according to the metabolomic analysis, we also measured the total fecal TG content biochemically, and found that it was higher in the HFMD group than in the ND and HFD groups (Figure 4d). The results of the lipid profiling of the lower digestive tract and fecal TG measurement imply that mushroom consumption promotes the excretion of lipids. Because an increase in insoluble dietary fiber has been reported to promote lipid excretion (Hsu et al., 2006; Neyrinck et al., 2009), these effects may be attributable to the fiber content of the mushrooms. However, the same amount of dietary fiber, in the form of cellulose and pectin, was added to the diet of the HFD group. Therefore, specific types of dietary fiber, such as 1,3-β-glucan, chitin, lignin, or cellulose which are present in mushroom content, may be present in the mushrooms, which may be responsible (Kurasawa et al., 1982). In addition to dietary fiber, the other compounds may participate in promotion of lipid excretion. It was reported that Pleurotus eryngii water extract has an effect greater synthesis of phospholipids found in the serum and liver likely reflect greater synthesis of lipoproteins, which are required for the transport of lipids and cholesterol around the body (Ockner et al., 1969), and which are also necessary for the maintenance of energy homeostasis in mice consuming mushrooms. It has been reported that a larger lipid concentration in the large intestine and feces disturbs the balance of the gut microbiome, which adversely affects the host (Murphy et al., 2015). However, we have previously reported that the gut microbial composition is altered by mushroom consumption, leading to an increase in the population of some SCFA- and lactic acid-producing bacteria (Shimizu et al., 2018), implying that the amount of TG in the feces is not likely to have adversely affected the gut microbiome.

3.5. Mushroom consumption increases the quantity of PC containing odd-chain fatty acids

Mushroom consumption increased the concentrations of some lipids containing odd-chain fatty acids (Tables 1, 2, 3, 4, 5, and 6). Figure 5 shows that PC containing odd-chain fatty acids was more abundant in the serum, liver, cecal contents, and feces of mice fed HFMD. Because long-chain fatty acids are synthesized from acetyl-CoA, they usually contain an even number of fatty acids. However, it is known that some gut bacteria produce propionic acid from water-soluble dietary fiber, which can be used to synthesize odd-chain fatty acids in the liver (Weitkunat et al., 2017). We hypothesize that the concentrations in the HFMD group increase following the metabolism of the water-soluble dietary fiber contained in the mushrooms by gut bacteria. Propionic acid produced by these bacteria promote energy metabolism in the host (Sarid, Poppelbosch, Roeofselen, Vonk and Venema, 2010). Therefore, we hypothesize that mushroom consumption increases the propionic acid concentration in the body, and that this plays a role in suppressing fat accumulation.
4. Conclusions

We aimed to examine how the mushroom consumption alters lipid metabolism in high-fat diet-fed mice. Mushroom consumption resulted in increased phospholipids in serum and liver, increased DG in adipose tissue, and increased TG in feces. The results showed that mushroom consumption promotes the excretion, rather than the absorption of dietary lipids through the gut. The mice that consumed the mushroom mixture were thus relatively energy-deficient and apparently maintained their energy homeostasis by using lipids from their visceral fat stores, reducing visceral fat mass. Thus, a lipid metabolomic analysis has elucidated part of the mechanism underpinning the suppression of fat accumulation by mushroom consumption.

Declarations

Author contribution statement

Takamitsu Shimizu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Koichiro Mori: Analyzed and interpreted the data; Wrote the paper.

Hitoshi Kobayashi: Analyzed and interpreted the data.

Tsuyoshi Tsuduki: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

Three of the authors (Takamitsu Shimizu, Koichiro Mori, and Hitoshi Kobayashi) are salaried employees of the Hokuto Corporation, which cultivated some of the mushrooms used in this study. The remaining author (Tsuyoshi Tsuduki) declares no conflict of interest. We used five types, 4 of which are from our company. However, 33% of the mixture represents a sample from another company. All research funding for this study was provided by the Hokuto Corporation.

Additional information

No additional information is available for this paper.

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