

Edible Mushrooms Reduce Atherosclerosis in Ldlr–/– Mice Fed a High-Fat Diet

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ABSTRACT

Background: Commonly consumed mushrooms, portobello (PBM) and shiitake (SHM), are abundant in nutrients, soluble dietary fibers, and bioactive compounds that have been implicated as beneficial in reducing inflammation, improving lipid profiles, and ameliorating heart disease and atherosclerosis, an inflammatory disease of the arteries.

Objective: The aim of this study was to determine effects of PBM and SHM in preventing atherosclerosis and associated inflammation in an animal model.

Methods: Four-week-old Ldlr-/- male mice were divided into 5 dietary groups for 16 wk: a low-fat control (LF-C, 11 kcal% fat), high-fat control (HF-C, 18.9 kcal% fat), HF + 10% (wt:wt) PBM (HF-PBM, 19.5 kcal% fat) or SHM (HF-SHM, 19.7 kcal% fat) powder, and HF + mushroom control mix (MIX-C, 19.6 kcal% fat), a diet best matched to the average macronutrient content of both mushrooms. Body composition was measured using MRI. Aortic tricuspid valves and aortas were collected and stained to quantify plaque formation. Adhesion molecule expression was quantified by immunohistochemistry. Plasma lipid and cytokine concentrations were measured.

Results: We found that mice fed a HF-SHM diet had ~86% smaller aortic lesion area than mice in both HF-C (P < 0.01) and MIX-C (P < 0.01) groups and also expressed 31–48% lower vascular cell adhesion molecule-1 levels (P < 0.05) than all other groups. Similarly, HF-PBM–fed mice displayed a 70% reduction in aortic lesion area in the tricuspid valve only (P < 0.05). Both mushroom-fed groups had lower weight gain and fat mass (P < 0.05) than the control groups.

Conclusion: These results suggest that consumption of PBMs and particularly SHMs is effective in preventing development of high-fat diet–induced atherosclerosis in Ldlr–/– mice. Future studies will determine active components in mushrooms responsible for this beneficial effect. *J Nutr* 2019;00:1–8.

Keywords: edible mushrooms, shiitake, portobello, atherosclerosis, heart disease, high-fat diet, LdIr-/- mice

Introduction

For centuries, mushrooms have been used as a medicinal food for various remedies. Over the past several decades, mushrooms have been recognized as a complementary food with many beneficial health effects (1). According to the FAO of the UN, edible mushroom production has doubled over a 10-y period from 1997 to 2007 (2). The most commonly grown mushrooms are Agaricus bisporus (common white and brown), Lentinula edodes (shiitake), and Pleurotus ostreatus (oyster). Mushrooms are good dietary sources of many micronutrients; along with chitins, they are great sources of both soluble and insoluble fiber. Soluble fiber, specifically β -glucan, is the major polysaccharide that reduces blood cholesterol. Dietary fiber in edible mushrooms may be one plausible mechanism by which mushrooms may contribute to reducing cardiovascular disease (CVD) risk. Further, certain bioactive components of mushrooms have anti-inflammatory, hypoglycemic, and antiatherogenic activities; therefore, mushrooms are anticipated

to improve CVD through their anti-inflammatory and antiatherogenic activities. According to limited preliminary data, in addition to the aforementioned components, several other components in edible mushrooms including antioxidants, low sodium, high potassium and selenium, B and D vitamins, and some bioactive compounds may benefit heart health (3). Thus, certain edible mushrooms are considered health-promoting functional foods.

Ergothioneine (ERG), a bioactive component of edible mushrooms, has been investigated for its antioxidant and anti-inflammatory activities as well as its potential role in suppression of atherosclerosis. Also, ERG has been reported to prevent lipid peroxidation in several tissues and cell types (4). Martin (5) reported that preincubation of human aortic endothelial cells with ERG suppressed IL-1 β -stimulated expression of adhesion molecules and reduced monocyte adhesion, the well-established hallmarks of inflammation and fatty streak development in the early stages of atherogenesis. These observations provided preliminary evidence for ERG's

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Manuscript received October 22, 2018. Initial review completed November 13, 2018. Revision accepted March 26, 2019. First published online 0, 2019; doi: https://doi.org/10.1093/jn/nxz075. potential to prevent inflammation and atherosclerosis. Although several types of mushrooms have been studied for their effects on serum lipid profile, few studies have demonstrated direct evidence supporting edible mushrooms' protective effect on atherogenesis. Current information on mushrooms' effect on atherosclerosis is limited to 3 rabbit studies (6-8) and 1 apoE-deficient (apoE-/-) mouse study (9). One study in rabbits showed reduced atheroma formation with a low dose of shiitake mushrooms (SHMs) without affecting blood cholesterol concentrations (6). In another rabbit study, oyster mushrooms were added to the diet of 5 rabbits/group (7). In that study, 2 animals in each group developed fatty streaks and 2 in the treated group developed fibromuscular plaque. The third rabbit study reported that supplementation of a highcholesterol diet with blushing wood mushroom (Agaricus sylvaticus) for 10 wk reduced oxidative stress and atheroma lesions (8). Finally, Mori et al. (9) reported that supplementing the "normal diet" (undefined) with 3 different mushrooms (eringi, maitake, and bunashimeji) reduced total cholesterol and atheroma lesions in apoE - / - mice. However, these mushrooms are not commonly consumed.

Based on the information reviewed above, we conducted the current study to determine the efficacy of 2 edible mushrooms, portobello mushroom (PBM) and shiitake mushroom (SHM), in the prevention of atherosclerosis. Several factors were considered in selecting these 2 species for their potential to prevent atherosclerosis. These mushrooms contain both soluble and insoluble fiber as well as bioactive compounds with antioxidant activity, such as ERG, which is suggested to have potential antiatherogenic activity as shown in cell culture systems and some preliminary animal studies. Among the A. bisporus species, PBMs contain higher amounts of ERG (0.45-0.72 mg/g dry weight) than the white button mushroom (0.41-0.47 mg/g dry weight), and the ERG content in SHM (1.98–2.09 mg/g dry weight) is \sim 4 times higher than in PBM (10, 11). Further, these mushrooms are commonly cultivated, consumed, and readily available at the market, and the public is aware of their overall general nutritive value. The SHM is the world's second-most consumed mushroom, only second to the white button mushroom. Taking all of the foregoing into consideration, we hypothesized that high fiber and the bioactive compounds in 2 edible mushrooms, PBM and SHM, would reduce inflammation and inhibit atherosclerosis development and progression. This hypothesis was tested in the current study using LDL receptor knockout (Ldlr-/-) mice as an animal model.

Methods

Mushroom powder preparation

PBMs and SHMs were generously donated by Phillips Mushroom Farms. Once received, they were immediately washed, the stalks were removed, and then the caps were chopped into small pieces. The mushrooms were then dried in a freeze dryer (Millrock Technology) for 3 d. The freeze-dried mushrooms were ground into a fine powder using a mixer and stored at -20° C before being mixed into the diet at 10% (wt:wt). A portion of the powders was sent to NP Analytical Laboratories (St. Louis, MO) for nutritional analysis of macronutrients and select micronutrients.

Animals and dietary intervention

A total of 60, 4-wk-old, male Ldlr-/- mice (Jackson Laboratory) were used in this study. All animal handling and procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Tufts University. Mice were singly housed in polycarbonate box cages and maintained in a specific pathogen-free barrier facility at 23°C under a 12-h light/dark cycle with ad libitum access to food and water. After 1 wk acclimation to a modified AIN-93M low-fat control diet (LF-C) (Teklad Laboratories), mice were randomly placed into 1 of the following 5 dietary treatment groups (n = 12/group) (Supplemental Table 1) and fed for 16 wk: 1) LF-C: basal AIN-93M LF-C that contained basal proportions of 4.5% by weight milk fat and soybean oil, making up 11% of total caloric intake; 2) high-fat control diet (HF-C): basal AIN-93M containing 8% by weight milk fat and soybean oil, making up 19% of total caloric intake; 3) HF-PBM: HF-C + 10% PBM powder; 4) HF-SHM: HF-C + 10% SHM powder; and 5) mushroom control diet (MIX-C): HF-C + 10% "control mixture" that was best matched to the average macronutrient composition of PBMs and SHMs and composed of 25% casein, 37.5% corn starch, and 37.5% cellulose. According to Wu et al. (12), 10% wt:wt mushroom powder in mice will translate to ~ 11 g fresh mushroom/kg body weight, which is ~ 750 g mushroom/d (10 servings/d) for a person with an average weight of 65-70 kg. This is higher than the recommended 5 servings of vegetables per day; however, we chose 10% freeze-dried powder of mushrooms to increase the probability of efficacy against lipid-induced atherosclerosis in mice. Body weight was measured weekly for 16 wk of the dietary treatment period. Before killing, body composition was determined using MRI (EchoMRI). After the conclusion of dietary treatment, mice were killed with carbon dioxide followed by exsanguination. Blood samples were collected in EDTA-coated tubes by heart puncture, and plasma was obtained by centrifugation and stored at -80° C for future analyses.

Tissue collection and processing

After killing, the chest cavity was opened and the heart and aorta were perfused with PBS. The heart and the descending aorta were dissected out. The apex of the heart was sectioned, snap-frozen, and stored at -80° C for later analysis. The aortic root containing the aortic tricuspid valve was dissected and separated from the aortic arc at the right subclavicular branching point, embedded in optimal cutting temperature compound, and frozen in liquid nitrogen for cryostat sectioning. For 3 mice/treatment group, a small section of the aortic arc was excised and collected in RNAlater solution (Qiagen) for RNA extraction and later RT-PCR analysis of expressed genes. The descending aorta was stored in 10% buffered formaldehyde solution for later *en face* staining.

Tissue staining

The descending aorta was opened longitudinally, pinned down on a wax platform, and stained with Oil Red O stain to visualize lesions as previously described (13). The total area of lesions was measured, and a ratio of lesion area to the perimeter of the vessel was established to account for differently sized vessels. Consecutive sections were stained with Oil Red O and hematoxylin for visualization of valves and plaque in the tricuspid valve. Sections were also fluorescently stained with vascular cell adhesion molecule-1 (VCAM-1) antibody

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Supplemental Table 1 and Supplemental Figure 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/. Address correspondence to MM (e-mail: mohsen.meydani@tufts.edu).

Abbreviations used: CVD, cardiovascular disease; ERG, ergothioneine; HF-C, high-fat control diet; HF-PBM, high-fat diet plus 10% portobello mushroom powder; HF-SHM, high-fat diet plus 10% shiitake mushroom powder; Ldlr, LDL receptor; LF-C, low-fat control diet; MIX-C, mushroom control diet; PBM, portobello mushroom; SHM, shiitake mushroom; VCAM-1, vascular cell adhesion molecule-1.



FIGURE 1 Body weights (A) and weight gains (B) in male LdIr–/– mice fed an LF-C, HF-C alone or supplemented with 10% PBM or 10% SHM powder, or MIX-C diet for 16 wk. Values are mean \pm SEM, n = 12. Labeled means without a common letter are significantly different, P < 0.05. *HF-SHM compared with LF-C and HF-C, P < 0.05. *HF-SHM compared with MIX-C, P < 0.05. ^HF-PBM compared with HF-C, P < 0.05. +HF-PBM compared with LF-C and MIX-C, P < 0.05. HF-C, high-fat control diet; HF-PBM, high-fat diet supplemented with 10% portobello mushroom powder; HF-SHM, high-fat diet supplemented with 10% shiitake mushroom powder; LF-C, low-fat control diet; MIX-C, mushroom control diet; PBM, portobello mushroom; SHM, shiitake mushroom.

(Invitrogen) to assess the state of inflammation in the valve. The intensity of fluorescent antibodies was compared with the total intensity of fluorescent nucleic acid stain by SYTOX Green Nucleic Acid Stain (Thermo Fisher Scientific) to account for differences in tissue thickness and then compared with the vessel perimeter.

Plasma inflammatory cytokines and lipid profile

Plasma total and HDL cholesterol and TGs were measured by an automated chemistry analyzer. Non-HDL cholesterol was calculated. Plasma concentrations of the inflammatory markers IL-1 β , IL-2, IL-5, IL-6, IL-10, IL-12p70, keratinocyte chemoattractant/human growth-regulated oncogene, TNF- α , and IFN- γ were measured by Meso Scale Discovery's Sector Imager system (Meso Scale Diagnostics).

Statistical analysis

All results were expressed as mean \pm SEM. The Shapiro–Wilk test was used to determine normality, and homogeneity of variance was evaluated using Bartlett's test before statistical analysis. Repeatedmeasures ANOVA (Group × Week) was used to analyze body weight of the 5 intervention groups and followed by Tukey's honestly significant difference (HSD) test to compare means between the groups. All other data were analyzed using 1-factor ANOVA followed by Tukey's honestly significant difference (HSD) post hoc test for multiple comparisons. Significance was determined at P < 0.05.



FIGURE 2 Body composition of male Ldlr–/– mice fed an LF-C, HF-C alone or supplemented with 10% PBM or 10% SHM powder, or MIX-C diet for 16 wk. Body lean and fat mass were measured by EchoMRI and percentages were calculated based on total weight measured before the test. Values are mean \pm SEM, n = 12. Labeled means without a common letter are significantly different, P < 0.05. Means were compared only with their respective measurement groups. HF-C, high-fat control diet; HF-PBM, high-fat diet supplemented with 10% portobello mushroom powder; LF-C, low-fat control diet; MIX-C, mushroom control diet; PBM, portobello mushroom; SHM, shiitake mushroom.

Results

Mushroom powder analysis

Results of chemical analysis of the freeze-dried mushroom powders showed that SHMs had higher proportions of insoluble, soluble, and total dietary fiber (39.6%, 2.37%, and 41.9%, respectively) than PBMs (23.7%, 1.0%, and 24.7%, respectively). SHMs also contained lower proportions of protein, fat, potassium, and sodium than did PBMs.

Body and tissue weight and body composition

At the end of the 16-wk intervention, both mushroomsupplemented groups had significantly lower body weights (P < 0.007) than the control groups (**Figure 1A**). Throughout the 16-wk intervention period, the mushroom groups displayed lower weight gain than the controls (P < 0.007) (Figure 1B). EchoMRI scans displayed a lower percentage body fat mass and higher percentage lean mass in both mushroom groups (P < 0.0007) (Figure 2). Whereas mushroom-treated groups had higher (P < 0.05) percentage lean mass than the HF-C group, only HF-SHM displayed greater percentage lean mass (P < 0.001) than the LF-C and MIX-C groups in addition to HF-C.

Effect of mushrooms on fatty streaks and plaque formation in the aortic tricuspid valve and descending aorta

Aortic lesion area was compared with the total area of the aorta. The HF-SHM group displayed significantly smaller lesion area in the descending aorta than did the HF-PBM, HF-C, and MIX-C groups (P < 0.05) (Figure 3). Similar results were found for plaque formation in the aortic tricuspid valve. The HF-SHM group also displayed a smaller lesion area than the HF-C and MIX-C groups (P < 0.0001) (Figure 4).

VCAM-1 expression in the aortic tricuspid valve

Increased adhesion molecule expression is a common occurrence in the pathogenesis of atherosclerosis. As expected, HF-C expressed higher levels of VCAM-1 than the other groups (**Figure 5**). HF-SHM mice had significantly lower expression of VCAM-1 in the aortic tricuspid valve (P < 0.05) than any other group.

Mushroom supplementation lowered total plasma lipids (cholesterol and TGs)

Mushroom-supplemented mice had lower plasma lipid concentrations than the other dietary groups. The HF-SHM and HF-PBM groups had significantly lower total cholesterol concentrations than the HF-C group (P < 0.0001) (Table 1). Both HF-C– and MIX-C–fed mice had high plasma concentrations of LDL cholesterol, which were greater than in both mushroom groups (P < 0.05). Interestingly, HDLcholesterol concentrations were similar in all groups except the HF-SHM group, in which it was lower (P < 0.05).

Inflammatory cytokines

HF-C-fed mice had significantly higher (P < 0.05) concentrations of TNF- α than all the other treatment groups (Supplemental Figure 2).

Discussion

In Western societies, atherosclerosis is the major underlying cause of mortality from CVD (14). It is a chronic inflammatory disease of the arteries, which is manifested through the formation of fatty fibrous plaques, a hallmark of atherosclerotic progression often associated with chronic inflammation and increased oxidative stress. Apart from the inherent causes of atherosclerosis, studies have shown that modulation of external factors, such as a healthy diet through dietary intervention, may prevent and reduce atherosclerotic risk (15-17). Thus, we chose to develop a dietary intervention study using PBM and SHM, 2 mushrooms widely known to be a nutritious, low-fat, and low-calorie food with various heart-healthy properties. The PBM and SHM species are 2 commonly consumed mushrooms that are readily available in many markets. Few animal studies have been conducted to elucidate the antiatherogenic benefits of these common mushrooms (6-9). To our knowledge, our study was the first to test the antiatherogenic properties of PBMs and SHMs using a high-fat- and high cholesterol-fed Ldlr-/- strain of mice, an appropriate model for human atherosclerosis due to its similarities in lipoprotein-cholesterol distribution to that of humans (18).

Our results demonstrated that atherosclerosis was successfully induced in Ldlr-/- mice as indicated by the formation of aortic lesions, elevated cholesterol concentrations, and increased inflammation. Dietary intervention with 10% mushroom powder in mice fed a high-fat diet decreased lesion formation, improved plasma lipid profiles, and reduced adhesion molecule expression. The reduction of lesion area measured in the descending aorta and aortic tricuspid valve may be attributed to less inflammation and improved lipid concentrations seen in HF-PBM and HF-SHM mice, despite being fed a diet closely matched in fat and cholesterol concentrations. A previous study found that 10% supplementation of SHM powder greatly improved serum lipid metabolite, insulin, and glucose concentrations in male rats (19). In our study, decreased



FIGURE 3 Visualization of lesions in aortic arch and descending aorta of male LdIr—/— mice fed an LF-C (A), HF-C (B), HF-PBM (C), HF-SHM (D), or MIX-C (E) diet for 16 wk, and statistical summary (F) of lesion severity in different groups. Lesions were determined by Oil Red O lipid staining. Red arrows point to lesion formations. Values are mean \pm SEM, n = 5–7. Labeled means without a common letter are significantly different, P < 0.05. HF-C, high-fat control diet; HF-PBM, high-fat diet supplemented with 10% portobello mushroom powder; HF-SHM, high-fat diet supplemented with 10% shiitake mushroom powder; LF-C, low-fat control diet; MIX-C, mushroom control diet.

lipids were accompanied by lower weight gain and improved body composition.

A study by Handayani et al. (20) concluded that SHMs might inhibit fat deposition in rats, potentially through increased fat elimination by the presence of β -glucan, β -Glucan, a soluble fiber, is an important component of mushrooms noted for its hypolipidemic properties in its ability to bind cholesterol in the gut and reduce cholesterol absorption (21). Other animal studies have also reported the benefits of β -glucan in improving heart health by reducing blood glucose, cholesterol, and TGs (22–24). The USDA National Nutrient Database (25) indicated that SHMs have higher soluble fiber content than PBMs. Analysis of SHM powder indicated a 2-fold higher soluble fiber content than PBMs, which may have contributed to the differential effects observed in atherosclerotic risk outcomes.

Given the above findings, we formulated the MIX-C diet in our study to control for total fiber content of mushrooms in order to isolate the potential effects of mushroom-derived specific fibers such as β -glucan, as well as other potential unmeasured bioactive components such as ERG, vitamin D, folate, magnesium, and potassium, on antiatherosclerotic measures. MIX-C-fed mice displayed no difference in these atherosclerotic parameters compared with the HF-C group, which indicates that the β -glucan, rather than any dietary fibers,



FIGURE 4 Histology of lesions in aortic tricuspid valve of male Ldlr-/- mice fed an LF-C (A), HF-C (B), HF-PBM (C), HF-SHM (D), or MIX-C (E) diet for 16 wk, and statistical summary (F) of lesion severity in different groups. Lesions were determined by hematoxylin and Oil Red O lipid staining. Red arrows point to lesion formations. Values are mean \pm SEM, n = 5. Labeled means without a common letter are significantly different, P < 0.05. HF-C, high-fat control diet; HF-PBM, high-fat diet supplemented with 10% portobello mushroom powder; HF-SHM, high-fat diet supplemented with 10% shiitake mushroom powder; LF-C, low-fat control diet; MIX-C, mushroom control diet.

together with bioactive components not present in the MIX-C diet may have contributed to the antiatherogenic effect of mushrooms. Further investigation is needed to determine the key components in mushroom which attenuated atherosclerotic risk due to high-fat diet.

In addition, mushrooms may improve high-fat dietinduced inflammation by reducing inflammatory cytokines and increasing anti-inflammatory cytokines. The cause of improved inflammatory markers is multifaceted but reduced inflammation may be due to the presence of the soluble fiber β -glucan, vitamins and minerals, and an understudied bioactive compound, ergothioneine. Several studies have shown an association between mushroom consumption and improved immune response by upregulation of both innate and adaptive immunity (12, 24, 26–28). Although limited studies have been conducted in humans, 1 clinical study observed an enhancement of Th1-phenotype immune function through the IL-12/TNF- α pathway in subjects who were fed oyster mushrooms for 8 wk (29). However, contrary to the proposed rationale, we did not find a significant effect of mushroom supplementation on plasma cytokine concentrations. Because there was no difference in plasma TNF- α between the HF-C group and either of the mushroom groups, it is possible that the antiatherogenic effect of mushrooms may not be mediated through modulating production of systemic proinflammatory cytokines.

On the other hand, mushroom supplementation reduced expression of the adhesion molecule VCAM-1, found primarily at sites of atheroma formation. VCAM-1 plays an imperative role in mediating leukocyte adhesion and migration under proatherogenic conditions, a detrimental process when prolonged in chronic inflammatory states (30, 31). Studies have linked VCAM-1–mediated inflammation to the activity of the proinflammatory cytokine TNF- α through activation of NF- κ B



FIGURE 5 VCAM-1 expression in aortic tricuspid valve of male Ldlr–/– mice fed an LF-C (A), HF-C (B), HF-PBM (C), HF-SHM (D), or MIX-C (E) diet for 16 wk, and statistical summary (F) of VCAM-1 expression in different groups. VCAM-1 expression was determined by immunohistochemistry. White arrows point to areas of high VCAM-1 expression. Values are mean \pm SEM, n = 7–11. Labeled means without a common letter are significantly different, P < 0.05. HF-C, high-fat control diet; HF-PBM, high-fat diet supplemented with 10% shiitake mushroom powder; LF-C, low-fat control diet; MIX-C, mushroom control diet; VCAM-1, vascular cell adhesion molecule-1.

(32–34). In our study, both mushrooms decreased TNF- α and VCAM-1 to levels similar to the LF-C group, indicating that the potential antiatherogenic effects of SHMs and PBMs may be due to downregulation of NF- κ B and Nrf2 pathways, which are involved in inflammation and cellular antioxidant status,

respectively (12, 35, 36). Further studies are needed to validate this association.

A limitation in the current study is that mice had free access to the diets and we did not measure the actual food intake. Addition of mushrooms to the diet may have potentially

TABLE 1 Plasma lipid profiles of male LdIr-/- mice fed an LF-C, HF-C alone or supplemented with 10% PBM or 10% SHM powder, or MIX-C diet for 16 wk¹

Parameter, mg/dL	LF-C	HF-C	HF-PBM	HF-SHM	MIX-C
Total cholesterol	455 ± 55.8 ^{b,c}	776 ± 234.9^{a}	366 ± 20.2 ^{c,d}	235 ± 23.4^d	$669 \pm 100.8^{a,b}$
LDL cholesterol	344 ± 49.1^{b}	$660~\pm~39.6^{a}$	$266 \pm 17.0^{\rm b,c}$	166 ± 21.0°	$548~\pm~88.6^{\rm a}$
HDL cholesterol	51.7 ± 4.64^{a}	53.3 ± 3.97^{a}	56.4 ± 2.29^{a}	32.9 ± 4.09^{b}	51.0 ± 3.40^{a}
TGs	$294~\pm~29.5^{a,b,c}$	$316~\pm~33.3^{a,b}$	$217~\pm~16.8^{b,c}$	$178~\pm~12.4^{\circ}$	$349~\pm~61.7^a$

¹Values are mean \pm SEM, n = 8-12. Labeled means in a row without a common letter are significantly different, P < 0.05. HF-C, high-fat control diet; HF-PBM, high-fat diet supplemented with 10% shiitake mushroom powder; LF-C, low-fat control diet; MIX-C, mushroom control diet.

affected the diet intake of mice in the treatment groups. In other animal studies in which animals were fed a normal-fat diet, body weight was not generally affected by increased consumption of mushroom (7, 9, 12), whereas our animals fed a high-fat diet supplemented with mushroom displayed a significant reduction in body weight gain. However, our data indicate that the effects observed in reducing atherosclerosis are not due to reduction in food intake in general. Although mice fed either type of mushrooms showed lower weight gain, those supplemented with SHMs had significantly less atherosclerotic lesion, as previous studies have shown that rats supplemented with SHMs exhibited reduced serum lipids, lipophilic antioxidant capacity, and fat deposition (19, 20) compared with those fed PBMs.

Overall, SHMs had a greater overall impact on atherosclerotic measures, possibly because SHMs contain higher concentrations of fiber and ERG than PBMs (11, 37). ERG, a unique bioactive component found ubiquitously in the mushroom species, has been found to be a potent antioxidant and potential antiatherogenic compound. Its transporter, organic cation transporter 1, is also widely expressed in many tissues and conserved across species (38-41). Whereas SHM contains no more than 2-fold fiber than PBM per 100 g dried weight, based on Dubost et al. (10), its ERG content is >4 times that found in PBM (1.98 \pm 0.11 compared with 0.45 \pm 0.03 mg/g dry weight). This finding signifies that ERG may be an important compound of interest in preventing atherosclerosis. Accordingly, there has been recent effort to understand how this food-derived antioxidant may function in several diseases, in order to elucidate the exact working mechanism of ERG and its effect on diseases such as atherosclerosis (42, 43).

Atherosclerosis is now recognized as not merely a condition of hyperlipidemia but also a chronic inflammatory disease. Our results suggest that consumption of PBMs and SHMs may be effective in preventing high-fat-induced atherosclerosis in Ldlr-/- mice through modulation of lipid metabolism and immune function. Our findings provide new insight into dietary components which may reduce the risk of atherosclerosis. Future studies should aim to elucidate the key components of mushroom which reduce atherosclerosis, as well as their underlying mechanisms.

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