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# 舞茸菌絲體及多醣體對餵食高脂肪高膽固醇飼料倉鼠高脂血症和組織氧化壓力的影響

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# Effects of mycelia and polysaccharides of *Grifola frondosa* on hyperlipidemia and oxidative stress in tissues of hamsters fed a high-fat and high-cholesterol diet

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**Abstract** The fruiting bodies of *Grifola frondosa* (*G. frondosa*) had been shown to improve hepatic cholesterol metabolism and hypercholesterolemia. The aim of this study was to investigate the effects of fermented *G. frondosa* mycelium (MGF) and its intracellular (MIP) and extracellular (MEP) polysaccharides on redox homeostasis in non-adipose tissues under hyperlipidemic conditions. Male hamsters received a high-fat, high-cholesterol (HFHC) diet with intragastric administration of cellulose, MGF, MIP, or MEP for 8 weeks, while those fed a control diet and intragastric administration of cellulose served as controls. The results showed that MGF and MIP significantly reduced both triglycerides and cholesterol, whereas MEP specifically reduced cholesterol. In the hearts, all three preparations decreased [thiobarbituric acid reactive substances (TBARS)] levels; however, MGF and MIP decreased the reduced glutathione (GSH) content and superoxide dismutase activity, respectively. In the lungs, all three preparations increased glutathione peroxidase activity, and MEP further reduced TBARS levels and increased total thiol content. In the kidneys, MEP increased TBARS levels. In the gastrocnemius muscles, all three preparations reduced TBARS levels; MGF and MIP lowered total thiol content, whereas MGF increased GSH content. Overall, MGF, MIP, and MEP alleviated HFHC-induced dyslipidemia and provided tissue-specific antioxidant protection. These findings suggest that fermented *G. frondosa* mycelium and its polysaccharides have potential as functional dietary supplements to prevent oxidative stress-related tissue damage in hyperlipidemia.

**Key words: *Grifola frondosa*, hyperlipidemia, polysaccharides, oxidative stress, antioxidant enzymes**

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# INTRODUCTION

Hyperlipidemia is a global public health concern that increases the risk of developing cardiovascular diseases [1]. The manifestation of excess circulating lipids is considerably influenced by factors such as inheritance and dietary patterns. Chronic consumption of high-fat and high-cholesterol (HFHC) diets is well-documented as a contributor to hyperlipidemia and organ-specific damage, with myocardial injury being the most affected. The accumulation of lipid peroxides in cardiomyocytes impairs antioxidant defenses and leads to structural and functional deterioration [2]. Increasing evidence also shows that hyperlipidemia causes damage across multiple organs and tissues, largely through oxidative stress, inflammation, mitochondrial dysfunction, and impaired antioxidant defense mechanisms [3, 4]. The endogenous enzymatic antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase, along with the glutathione system, can effectively eliminate reactive oxygen species, which are often overwhelmed under chronic hyperlipidemia. Consequently, oxidative stress and impaired antioxidant defense synergistically drive progressive tissue injury. Therefore, exploring hyperlipidemia-induced organ damage from the perspective of lipid peroxidation, oxidative stress, and antioxidant mechanisms provides critical insight into the pathogenesis of systemic complications associated with dyslipidemia.

There is increasing evidence that antioxidant-rich diets protect against chronic diseases, notably hyperlipidemia [5]. Edible mushrooms are valuable foods for humans and rich natural sources of antioxidants due to their bioactive compounds. Among these, polysaccharides from mushrooms have attracted considerable attention for their broad physiological activities. For example, polysaccharides from species such as *Cordyceps militaris* and *Ganoderma lucidum* have been shown to exert anti-hyperglycemic, anti-hyperlipidemic, and organ-protective effects, largely through modulation of oxidative stress related pathways [6, 7]. *Grifola frondosa* (*G. frondosa*) is well recognized for its immunomodulatory, antitumor, and antidiabetic properties [8]. Our previous work demonstrated that polysaccharides derived from fermented *G. frondosa* mycelia and fermentation broth effectively ameliorated hypercholesterolemia and oxidative stress in the erythrocytes and liver of HFHC-fed hamsters [9].

Despite these promising findings, few studies have directly compared polysaccharides from fermented *G. frondosa* mycelia (MGF), particularly those isolated from both intracellular (MIP) and extracellular (MEP) fractions, with respect to their lipid-lowering and antioxidant effects. Furthermore, the tissue-specific modulation of hyperlipidemia-associated oxidative stress by these distinct polysaccharide fractions has yet to be systematically investigated. In this study, we hypothesized that hyperlipidemia-induced oxidative stress and redox imbalance triggered by a HFHC diet could be mitigated by polysaccharide-rich preparations from *G. frondosa*. To test this hypothesis, we systematically evaluated the effects of MGF, MIP, and MEP on HFHC-induced lipid abnormalities and on biomarkers of lipid peroxidation and antioxidant defense in the lungs, heart, kidneys, and gastrocnemius muscles of hamsters.

# MATERIALS AND METHODS

## Preparation of Fermented Mycelia and Polysaccharide Fractions of *G. frondosa*

*G. frondosa* (BCRC 36434; Bioresources Collection & Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan) was cultured to obtain fermented mycelia and broth, following procedures adapted from our earlier work [9]. The strain was grown in a 30-L bioreactor (Top-Bio, Taiwan) containing fermentation medium (w/v: 2% glucose, 1% soy hydrolysate, 1% yeast extract, 0.1% $KH_2PO_4$, 0.1% $K_2HPO_4$ and 0.1% $MgSO_4 \cdot 7H_2O$; pH 5.0 before sterilization). Cultivation was initiated with

a 10% inoculum at 25℃, aerated at 1 vvm, and stirred at 200 rpm, gradually increasing to 350–400 rpm. After 7 days, the biomass was separated by filtration, washed thoroughly with distilled water, and dried at 50℃ to yield mycelium (MGF).

The mycelial biomass and fermentation broth were used to extract MIP and MEP, respectively [10]. For MIP extraction, dried MGF was mixed with distilled water (1:10, w/v) and autoclaved at 121℃ for 30 minutes. The extract was filtered, mixed with four volumes of 95% ethanol, and incubated at 4℃ overnight. The precipitate was collected by centrifugation, washed three times with distilled water, and freeze-dried for 48 hours. For MEP, dried broth powder was mixed with 95% ethanol (1:3, w/v), extracted overnight at 4℃, centrifuged, and freeze-dried. All dried samples were reconstituted in distilled water before administration. The polysaccharide contents, determined by the phenol-sulfuric acid assay, were 48.4 mg/g in the mycelial biomass and 1.7 mg/mL in the broth.

## Animals and Experimental Design

Thirty male hamsters (6 weeks old) were obtained from the National Laboratory Animal Center (Taipei, Taiwan). The study protocol was approved by the Laboratory Animal Care and Use Committee of Changhua Christian Hospital, Changhua, Taiwan (Approval No. CCH-AE-94004). Animals were housed under controlled conditions with free access to water and chow (AIN-76, MP Biomedicals, Irvine, CA, USA) during acclimatization. Hamsters were randomly assigned to 5 groups (n = 6/group). The control group (CON) received a cholesterol-free chow diet containing 5% fat (corn oil, 11.7% of total calories). Four hyperlipidemic groups were fed a HFHC diet containing 15% fat (w/w), comprising 10% corn oil and 5% coconut oil, corresponding to approximately 32% of total caloric intake. Cholesterol was included at 0.5% (w/w). The percentage of saturated and unsaturated fatty acids in the corn oil (MP Biomedicals, 901414) and coconut oil (MP Biomedicals, 901403) were 13.3:85.5 and 91.4:8.0, respectively. The HFHC groups were administered MGF, MIP, or MEP by daily intragastric gavage, whereas the CON and HFHC control (HTC) groups received an equivalent amount of cellulose. The estimated daily polysaccharide intake (~50 mg) was matched among MGF, MIP and MEP groups. Body weight and food intake were recorded weekly. Blood samples were collected from the retro-orbital sinus after overnight fasting at baseline, day 29, and day 43. On day 57, hamsters were anesthetized with ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight), and blood, lungs, hearts, kidneys, and gastrocnemius muscles were collected, weighted, and snap-frozen for subsequent analyses.

## Analytical Measurements

Serum triglyceride (TG) and total cholesterol (TC) were determined using commercial assay kits (Fortress Diagnostics Limited, Antrim, UK). The marker of oxidative stress, i.e., thiobarbituric acid reactive substances (TBARS), and antioxidant defenses in tissue homogenates were determined as described in our previous study [9]. Total thiol groups were measured by using Ellman's reagent following the method of Sedlak and Lindsay [11]. Both reduced glutathione (GSH) and oxidized glutathione (GSSG) were fluorometrically based on the method of Hissin and Hilf [12]. The activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase in tissue homogenates were evaluated. SOD activity was expressed in pyrogallol units based on inhibition of pyrogallol autoxidation [9]. GPx activity was expressed as μmol nicotinamide adenine dinucleotide phosphate (NADPH) oxidized/min/gram tissue. Catalase activity was measured spectrophotometrically by monitoring hydrogen peroxide ($H_2O_2$) decomposition and expressed as nmol $H_2O_2$ degraded/min/gram tissue.

## Statistical Analysis

Data are expressed as the means ± standard error of the mean (SEM). One-way ANOVA followed by the least significant difference (LSD) *post hoc* test was used to compare differences among groups. Analyses

were conducted using SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA), and differences were considered significant at $P < 0.05$.

## RESULTS AND DISCUSSION

### *G. frondosa* mycelium and polysaccharides showed no significant effects on body or organ weights in HFHC-fed hamsters

The body and organ weights are shown in Table 1. No significant differences in body weight were observed among the groups, indicating that ingestion of *G. frondosa* mycelium (MGF) or its intracellular (MIP) and extracellular (MEP) polysaccharides did not affect body weight in HFHC-fed hamsters. In contrast, Jiang *et al*. reported that mice fed a diet providing 60% of total calories from fat exhibited significant body weight gain, which was markedly suppressed by 10-week ingestion of *G. frondosa* fruiting bodies [13]. This discrepancy may be attributed to differences in both diet composition and the test materials. The HFHC diet used in the present study contained a lower fat proportion (32% of total calories), likely to result in smaller, non-significant weight changes. Moreover, the fermented mycelium and especially its polysaccharide fractions (MIP and MEP) contain little or no fiber and lack many of the secondary metabolites present in fruiting bodies, which may limit their impact on body weight regulation. Similarly, there were no significant differences in the weights of the hearts, lungs, kidneys, or gastrocnemius muscles among the groups, suggesting that these preparations did not induce organ hypertrophy or atrophy under the present dietary conditions.

### *G. frondosa* polysaccharides attenuated HFHC-induced hyperlipidemia

The HFHC diet induced a significant increase in serum TG levels in the HTC on day 29 and day 43 compared with the CON group (Fig. 1A). The increase was significantly alleviated in the MGF and MIP groups, with the TG-lowering effect of MIP being sustained until day 57. Similarly, serum TC levels in the HTC group were elevated by the HFHC diet on day 29, day 43, and day 57 compared with the CON group (Fig. 1B). This HFHC-induced hypercholesterolemia was significantly reduced in the MGF group on day 29, and in the MGF, MIP, and MEP groups on day 57.

The anti-hyperlipidemic effects of polysaccharides from medicinal mushroom fruiting bodies have been widely studied [14-16]. In this study, fermented *G. frondosa* mycelia (MGF) and its intracellular polysaccharides (MIP) more effectively reduced serum

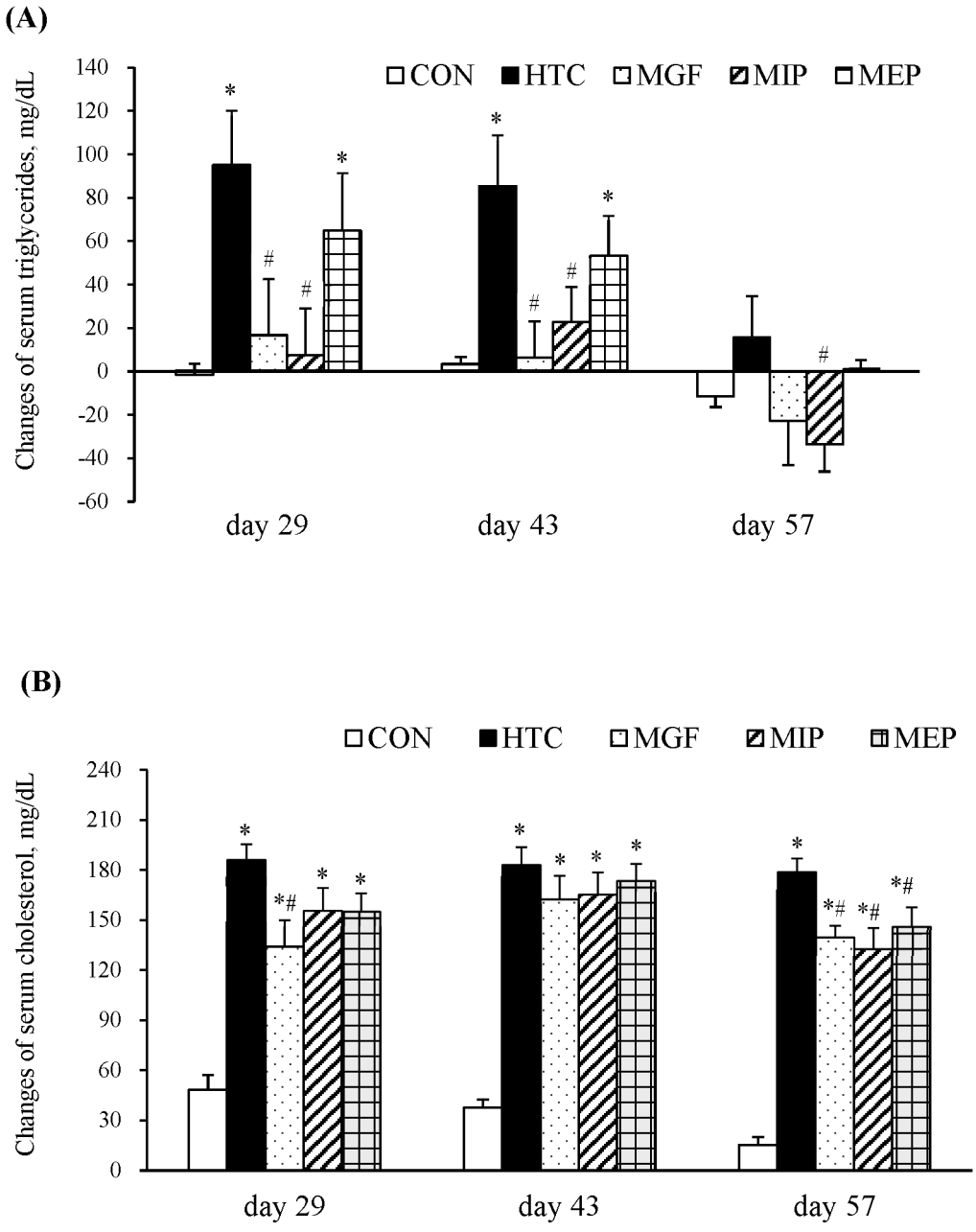
表一　倉鼠體重與器官重量。

**Table 1.** Body and organ weights of control and HFHC-fed hamsters

| | | Hyperlipidemic groups | | | |
|---|---|---|---|---|---|
| Groups | CON | HTC | MGF | MIP | MEP |
| Initial body weight, g | 81.3±4.3 | 83.2±3.8 | 81.3±3.7 | 82.5±3.3 | 80.3±3.7 |
| Final body weight, g | 98.4±3.2 | 103.9±5.2 | 101.0±2.7 | 103.4±0.8 | 101.8±3.5 |
| Heart weight, g | 0.38±0.01 | 0.37±0.03 | 0.40±0.01 | 0.38±0.01 | 0.40±0.03 |
| Lung weight, g | 0.57±0.02 | 0.60±0.02 | 0.57±0.02 | 0.59±0.02 | 0.61±0.02 |
| Kidney weight, g | 0.85±0.02 | 0.77±0.06 | 0.85±0.03 | 0.76±0.02 | 0.75±0.02 |
| Muscle weight, g | 0.39±0.02 | 0.41±0.04 | 0.35±0.04 | 0.37±0.01 | 0.42±0.02 |

Values are means±SEM, n = 6. Group means are analyzed using one-way ANOVA followed by least significant difference.

Abbreviations: CON, control; HTC, hyperlipidemic control; MGF, fermented mycelia of *Grifola frondosa*; MIP, intracellular polysaccharides extracted from fermented mycelia of *Grifola frondosa*; MEP, extracellular polysaccharides extracted from fermented broth of *Grifola frondosa*.




圖一　舞茸菌絲體及其多醣體對倉鼠血清 (A) 三酸甘油酯和 (B) 總膽固醇的影響。

**Figure 1.** Changes in serum (A) triglyceride and (B) total cholesterol concentrations from baseline (day 0) to days 29, 43, and 57. Values are means ± SEM (n = 6). *P < 0.05 vs. CON; #P < 0.05 vs. HTC (one-way ANOVA followed by LSD test).

TG and TC levels than extracellular polysaccharides (MEP) under HFHC feeding, likely due to differences in monosaccharide composition, bioactive constituents, or polysaccharide-mediated metabolic regulation [8]. According to our previous study, glucose was the predominant monosaccharide in all three preparations, while MIP contained a relatively higher proportion of arabinose (13.5%), and MEP was enriched in galactose (12.4%), mannose (11.3%), and rhamnose (4.5%) [9]. The high arabinose content in MIP may contribute to its antioxidant activity and tissue-specific protective effects. Supporting evidence indicated that arabinose-containing polysaccharides can modulate oxidative stress and chronic inflammation [17].

Moreover, Song *et al*. reported that L-arabinose can alleviate oxidative stress in LLC-PK1 cells exposed to high glucose [18].

Hyperlipidemia is well established as a key contributor to multi-organ injury, including pulmonary dysfunction [19], cardiac oxidative stress [20], impaired hepatic lipid metabolism [9, 21], and kidney injury [22]. This study examined the effects of fermented *G. frondosa* mycelia and polysaccharides isolated from mycelium and broth fractions on organs with distinct metabolic and physiological characteristics, including high oxygen-demand tissues such as the lungs and kidneys, as well as the heart and skeletal muscles, which are primarily composed of dense muscular tissue. We also evaluated tissue-specific lipid-lowering and antioxidant effects under hyperlipidemic stress. In the following sections, we discuss the differential impacts of *G. frondosa* polysaccharides on lipid metabolism, oxidative stress, and organ-specific responses.

## *G. frondosa* mycelium and polysaccharides reduced cardiac lipid peroxidation and differentially modulated antioxidant parameters in HFHC-fed hamsters

Hyperlipidemia is recognized as a risk factor for cardiovascular diseases, and lipid peroxidation is considered one of the earliest detectable events in the pathogenesis of atherosclerosis [23]. In this study, cardiac TBARS levels were not significantly different between the CON and HTC groups; however, were significantly reduced in the MGF ($P = 0.007$), MIP ($P < 0.001$), and MEP ($P < 0.001$) groups compared with the HTC group (Table 2). In addition, the MGF group exhibited a significant decrease in GSH content ($P = 0.037$) and GSH/GSSG ratios ($P = 0.012$) compared with the HTC group. The MIP group showed a marked reduction in SOD activity ($P < 0.001$) compared with the HTC group. GPx activity was significantly elevated in the MGF ($P = 0.018$) and MIP ($P = 0.023$) groups compared to the CON group.

No significant differences in lipid peroxidation or antioxidant indices were observed between the CON and HTC groups. This contrasts with findings from Noeman *et al.*, who reported that obese rats fed a high-fat diet (20.5% beef tallow and 25.5% corn oil) for 16 weeks exhibited increased cardiac malondialdehyde levels and reduced glutathione S-transferase and GPx activities [21]. Such discrepancies may be attributed to differences in dietary fat composition, saturated fat levels, and feeding duration. Our HFHC diet, containing 32% of total calories from fat (equivalent to 15% by weight) for 8 weeks, may not have been sufficient to induce pronounced oxidative stress in cardiac tissue. Additionally, the inherently strong antioxidant capacity of the heart may have mitigated potential oxidative effects, resulting in the absence of detectable changes in the HTC group.

Although oxidative stress was not induced in the HTC group, all three preparations effectively attenuated cardiac lipid peroxidation in HFHC-fed hamsters, with distinct effects on antioxidant defenses. MGF primarily reduced TBARS levels by modulating the GSH system, a key lipid peroxide scavenger and central endogenous antioxidant in the cardiovascular system [20], likely to enhance glutathione cycling and transiently lower the GSH/GSSG ratio. In addition, both MGF and MIP exhibited higher GPx activity than the CON group, suggesting an upregulation of enzymatic antioxidant defense. In contrast, MIP decreased SOD activity, suggesting a compensatory shift toward GPx-mediated antioxidant defense in cardiac tissue. GPx eliminates $H_2O_2$ and lipid peroxides, providing more effective defense than SOD, as elevated SOD can increase $H_2O_2$ accumulation [24]. This interpretation is supported by previous findings showing that a high-fat diet decreased SOD activity in rat cardiac muscle while GPx activity remained unchanged [25].

Besides, the improved systemic lipid profiles of MGF and MIP may also contribute to the reduction of cardiac oxidative stress and modulate downstream antioxidant responses. Collectively, *G. frondosa* mycelia (MGF) and its intracellular polysaccharides (MIP) exhibited cardio-protection under hyperlipidemic stress.

表二　舞茸菌絲體及其多醣體對倉鼠心臟組織之脂質過氧化物與抗氧化指標的影響。

**Table 2.** Lipid peroxidation and antioxidant parameters in heart tissues of hamsters

| | | Hyperlipidemic groups | | | |
|---|---|---|---|---|---|
| Groups | CON | HTC | MGF | MIP | MEP |
| TBARS, nmol/g tissue | 359.3±14.7 | 392.1±13.0 | 337.1±17.3$^{\#}$ | 300.0±10.6*$^{\#}$ | 307.4±9.3*$^{\#}$ |
| Total thiol groups, μmol/g tissue | 4.18±0.85 | 5.30±1.03 | 4.68±0.62 | 3.78±0.96 | 5.04±0.83 |
| GSH, μg/g tissue | 533.3±25.4 | 601.0±14.1 | 513.1±44.7$^{\#}$ | 539.2±23.2 | 605.9±24.7 |
| GSSG, μg/g tissue | 333.0±14.3 | 373.9±27.3 | 411.7±20.0* | 360.1±24.5 | 371.9±23.9 |
| GSH/GSSG ratio | 1.61±0.08 | 1.65±0.11 | 1.26±0.13*$^{\#}$ | 1.52±0.07 | 1.65±0.10 |
| SOD, unit/mg tissue | 16.1±1.4 | 15.1±2.6 | 14.3±1.8 | 3.1±0.8*$^{\#}$ | 13.7±1.8 |
| GPx, μmole NADPH/ min/g tissue | 5.94±0.28 | 6.86±0.31 | 7.15±0.31* | 7.09±0.45* | 6.24±0.32 |
| Catalase, nmol $H_2O_2$/ min/g tissue | 11.8±0.7 | 11.2±0.3 | 11.3±0.2 | 12.0±0.2 | 11.4±0.2 |

Values are means ± SEM (n = 6). *P < 0.05 vs. CON; $^{\#}$P < 0.05 vs. HTC (one-way ANOVA followed by LSD test). Abbreviations: CON, control; HTC, hyperlipidemic control; MGF, fermented mycelia of *Grifola frondosa*; MIP, intracellular polysaccharides extracted from fermented mycelia of *Grifola frondosa*; MEP, extracellular polysaccharides extracted from fermented broth of *Grifola frondosa*; TBARS, thiobarbituric acid reactive substances; GSH, glutathione; GSSG, oxidized glutathione; SOD, superoxide dismutase; GPx, glutathione peroxidase; NADPH, nicotinamide adenine dinucleotide phosphate; $H_2O_2$, hydrogen peroxide.

## *G. frondosa* mycelium and polysaccharides modulated oxidative stress markers in the lungs of HFHC-fed hamsters

Dyslipidemia is increasingly recognized as a contributor to pulmonary disease, partly due to lipid accumulation in alveolar epithelial cells, which induces oxidative stress and inflammation [19]. As the primary organ for gas exchange, the lungs are continuously exposed to high oxygen tension, making them particularly susceptible to oxidative stress. In this study, HFHC feeding did not significantly change lung TBARS levels in the HTC group compared with the CON group (Table 3) but significantly decreased GSH content (P = 0.029) and the GSH/GSSG ratios (P = 0.039). MGF and MIP groups exhibited higher TBARS levels than the CON group and increased GPx activity compared with HTC. GSH content was lower in MIP (P = 0.042) and MEP (P = 0.026) than in CON. In contrast, MEP reduced TBARS levels (P = 0.012) and increased total thiol content (P = 0.007) and GPx activity (P < 0.001) relative to HTC. GSSG content and SOD and catalase activities showed no significant differences among groups.

In pulmonary tissue, MEP increased total thiol groups and GPx activity while decreasing GSH levels, suggesting that MEP modulates the GSH system and upregulates non-glutathione sulfur-containing proteins, thereby mitigating lipid peroxidation. In contrast, MGF and MIP enhanced GPx activity without affecting TBARS levels, which may be associated with their monosaccharide composition, particularly their lower mannose and galactose content [9]. These results are consistent with the study by Xiang *et al*., which reported that exopolysaccharides from *Inonotus obliquus* containing higher mannose (24.8%) exhibited stronger antioxidant activity [26]. Notably, such modulations of antioxidative status were not observed in the HTC group, in which pulmonary GSH levels and GSH/GSSG ratios were markedly decreased. Collectively, these findings indicate that MGF, MIP and MEP differ

表三　舞茸菌絲體及其多醣體對倉鼠肺臟組織之脂質過氧化物與抗氧化指標的影響。

**Table 3.** Lipid peroxidation and antioxidant parameters in lung tissues of hamsters

| | | Hyperlipidemic groups | | | |
|---|---|---|---|---|---|
| Groups | CON | HTC | MGF | MIP | MEP |
| TBARS, nmol/g tissue | 482.5±17.3 | 585.2±23.3 | 626.7±73.6* | 609.9±48.1* | 422.7±21.5$^{\#}$ |
| Total thiol groups, μmol/g tissue | 4.46±0.55 | 3.29±0.56 | 5.09±0.95 | 3.80±0.70 | 7.85±2.00*$^{\#}$ |
| GSH, μg/g tissue | 843.0±157.7 | 494.6±97.1* | 615.0±78.9 | 520.3±121.0* | 486.2±38.7* |
| GSSG, μg/g tissue | 459.9±68.0 | 436.5±15.7 | 434.1±15.1 | 384.6±32.3 | 396.3±19.7 |
| GSH/GSSG ratio | 1.93±0.43 | 1.13±0.22* | 1.42±0.17 | 1.30±0.25 | 1.23±0.09 |
| SOD, unit/mg tissue | 6.69±0.86 | 5.73±0.64 | 6.30±0.38 | 5.94±0.84 | 6.13±0.39 |
| GPx, μmole NADPH/ min/g tissue | 6.62±0.27 | 6.31±0.24 | 7.41±0.25$^{\#}$ | 7.39±0.25$^{\#}$ | 8.23±0.42*$^{\#}$ |
| Catalase, nmol $H_2O_2$/ min/g tissue | 2.93±0.25 | 2.71±0.26 | 3.10±0.32 | 3.45±0.30 | 3.19±0.23 |

Values are means ± SEM (n = 6). *P < 0.05 vs. CON; $^{\#}$P < 0.05 vs. HTC (one-way ANOVA followed by LSD test). Abbreviations: CON, control; HTC, hyperlipidemic control; MGF, fermented mycelia of *Grifola frondosa*; MIP, intracellular polysaccharides extracted from fermented mycelia of *Grifola frondosa*; MEP, extracellular polysaccharides extracted from fermented broth of *Grifola frondosa*; TBARS, thiobarbituric acid reactive substances; GSH, glutathione; GSSG, oxidized glutathione; SOD, superoxide dismutase; GPx, glutathione peroxidase; NADPH, nicotinamide adenine dinucleotide phosphate; $H_2O_2$, hydrogen peroxide.

in their effects on lung redox homeostasis, with MEP uniquely enhancing total thiol reserves and promoting GSH utilization to alleviate pulmonary lipid peroxidation. Further studies are required to elucidate the specific monosaccharide profile of MEP on pulmonary protection.

## *G. frondosa* mycelium and polysaccharides modulated oxidative stress markers in the kidneys of HFHC-fed hamsters

The kidneys play a vital role in excretory function and are highly susceptible to metabolic disturbances. Dyslipidemia has been implicated in the development and progression of renal injury [(22)]. Therefore, excess lipid accumulation in the intrarenal microenvironment may initiate glomerular damage involving oxidative stress and inflammatory responses.

In the present study, renal TBARS levels were significantly higher in MGF (P = 0.012) and MEP (P < 0.001) than in CON, and MEP increased TBARS compared with HTC (P = 0.005) (Table 4). GSH was significantly higher in MGF (P = 0.039) than in CON, while GSSG was elevated in MGF (P = 0.045) and MIP (P = 0.039) compared with CON. GPx activity was significantly higher in MIP than CON (P = 0.013). No significant differences were observed among groups for total thiols, the GSH/GSSG ratios, SOD, or catalase.

The kidney possesses the capacity for concurrent GSH synthesis, degradation, efflux, and uptake [(27)], which is critical for maintaining redox homeostasis. However, hyperlipidemia can induce renal injury via lipid accumulation and increase oxidative stress [(22)]. In this study, renal TBARS level was significantly elevated in the MEP group compared to the HTC groups, potentially associated with hyperlipidemic conditions. In contrast, MGF and MIP, but not MEP, significantly reduced serum TG levels (Fig. 1A), suggesting that the observed differences in renal lipid peroxidation may be related, at least in part, to the lipid-lowering effects of

表四　舞茸菌絲體及其多醣體對倉鼠腎臟組織之脂質過氧化物與抗氧化指標的影響。

**Table 4.** Lipid peroxidation and antioxidant parameters in kidney tissues of hamsters

| | | Hyperlipidemic groups | | | |
|---|---|---|---|---|---|
| Groups | CON | HTC | MGF | MIP | MEP |
| TBARS, nmol/g tissue | 461.3±37.5 | 641.4±32.6 | 722.6±54.8* | 614.7±77.7 | 936.8±108.7*# |
| Total thiol groups, μmol/g tissue | 11.0±0.9 | 11.5±0.8 | 9.5±1.6 | 10.8±1.5 | 10.3±0.7 |
| GSH, μg/g tissue | 723.7±29.2 | 838.9±39.5 | 846.5±32.4* | 794.2±50.5 | 764.1±44.2 |
| GSSG, μg/g tissue | 695.8±31.2 | 775.8±47.3 | 809.8±28.0* | 813.7±48.6* | 748.2±30.6 |
| GSH/GSSG ratio | 1.05±0.05 | 1.09±0.04 | 1.05±0.03 | 0.98±0.03 | 1.02±0.05 |
| SOD, unit/mg tissue | 0.93±0.04 | 0.99±0.03 | 1.03±0.05 | 1.01±0.07 | 0.97±0.06 |
| GPx, μmole NADPH/ min/g tissue | 34.5±1.9 | 39.1±2.2 | 43.1±4.0 | 46.9±4.0* | 36.9±3.6 |
| Catalase, nmol $H_2O_2$/ min/g tissue | 4.11±0.20 | 4.26±0.29 | 4.16±0.25 | 4.05±0.26 | 4.33±0.36 |

Values are means ± SEM (n = 6). *P < 0.05 vs. CON; #P < 0.05 vs. HTC (one-way ANOVA followed by LSD test). Abbreviations: CON, control; HTC, hyperlipidemic control; MGF, fermented mycelia of *Grifola frondosa*; MIP, intracellular polysaccharides extracted from fermented mycelia of *Grifola frondosa*; MEP, extracellular polysaccharides extracted from fermented broth of *Grifola frondosa*; TBARS, thiobarbituric acid reactive substances; GSH, glutathione; GSSG, oxidized glutathione; SOD, superoxide dismutase; GPx, glutathione peroxidase; NADPH, nicotinamide adenine dinucleotide phosphate; $H_2O_2$, hydrogen peroxide.

*G. frondosa* polysaccharides. Moreover, GPx activity in the MIP group was significantly higher than that in the CON group, indicating that the polysaccharide can enhance renal antioxidant defenses even in the absence of detectable changes in TBARS level. These results support the notion that MGF and MIP may protect the kidney primarily by promoting GPx activity and facilitating glutathione turnover.

## *G. frondosa* mycelium and polysaccharides attenuated lipid peroxidation in gastrocnemius muscles of HFHC-fed hamsters

The HTC group exhibited significantly higher TBARS levels than CON (P = 0.004), indicating that the HFHC diet induced lipid peroxidation in gastrocnemius muscles (Table 5). Administration of MGF (P < 0.001), MIP (P < 0.001), and MEP (P = 0.004) significantly reduced TBARS compared with HTC, suggesting mitigation of lipid peroxidation by *G. frondosa* mycelium and polysaccharides. Both MGF (P = 0.046) and MIP (P = 0.026) significantly decreased total thiol groups compared with HTC. MGF also increased GSH level (P = 0.029 vs. CON; P = 0.008 vs. HTC) and GSH/GSSG ratios (P = 0.017 vs. CON; P = 0.004 vs. HTC) compared to CON and HTC, while MEP increased GSH/GSSG ratios compared with HTC (P = 0.035). No significant differences were found in GSSG, SOD, GPx, or catalase among groups.

As mentioned above, hyperlipidemia can increase lipid accumulation in peripheral non-adipose tissues, including skeletal muscle, driven by elevated fatty acid uptake and reduced mitochondrial lipid oxidation [28]. Excess lipid accumulation may lead to lipid peroxidation, generating reactive aldehydes such as 4-hydroxy-2-nonenal (4-HNE) that promote oxidative stress and impair cellular function. Elevated intramuscular lipid and 4-HNE levels have been linked to insulin resistance and metabolic dysfunction [29]. Antioxidant defenses can counteract these effects, as

表五　舞茸菌絲體及其多醣體對倉鼠腓腸肌之脂質過氧化物與抗氧化指標的影響。

**Table 5.** Lipid peroxidation and antioxidant parameters in gastrocnemius muscles of hamsters

| | | Hyperlipidemic groups | | | |
|---|---|---|---|---|---|
| Groups | CON | HTC | MGF | MIP | MEP |
| TBARS, nmol/g tissue | 203.7±19.1 | 325.2±28.6* | 158.6±16.7$^{\#}$ | 158.5±19.6$^{\#}$ | 205.4±41.9$^{\#}$ |
| Total thiol groups, μmol/g tissue | 5.62±0.62 | 7.45±0.79 | 4.94±0.84$^{\#}$ | 4.63±0.95$^{\#}$ | 6.82±0.99 |
| GSH, μg/g tissue | 100.9±8.1 | 94.9±9.6 | 127.1±6.4*$^{\#}$ | 92.7±4.2 | 107.6±10.0 |
| GSSG, μg/g tissue | 161.3±12.5 | 161.8±8.9 | 150.8±11.4 | 158.7±11.3 | 137.7±4.9 |
| GSH/GSSG ratio | 0.64±0.07 | 0.59±0.06 | 0.86±0.05*$^{\#}$ | 0.60±0.06 | 0.78±0.06$^{\#}$ |
| SOD, unit/mg tissue | 4.96±0.33 | 4.83±0.30 | 5.08±0.34 | 5.25±0.17 | 5.19±0.41 |
| GPx, μmole NADPH/ min/g tissue | 8.47±0.91 | 8.03±0.25 | 6.93±0.37 | 7.15±0.38 | 7.53±0.63 |
| Catalase, nmol $H_2O_2$/ min/g tissue | 0.67±0.09 | 0.82±0.12 | 0.72±0.07 | 0.64±0.06 | 0.91±0.13 |

Values are means ± SEM (n = 6). *P < 0.05 vs. CON; $^{\#}$P < 0.05 vs. HTC (one-way ANOVA followed by LSD test). Abbreviations: CON, control; HTC, hyperlipidemic control; MGF, fermented mycelia of *Grifola frondosa*; MIP, intracellular polysaccharides extracted from fermented mycelia of *Grifola frondosa*; MEP, extracellular polysaccharides extracted from fermented broth of *Grifola frondosa*; TBARS, thiobarbituric acid reactive substances; GSH, glutathione; GSSG, oxidized glutathione; SOD, superoxide dismutase; GPx, glutathione peroxidase; NADPH, nicotinamide adenine dinucleotide phosphate; $H_2O_2$, hydrogen peroxide.

demonstrated in vitro where enhanced antioxidant capacity prevented 4-HNE–induced insulin signaling defects [30].

Consistent with these reports, we observed that the HTC group exhibited elevated TBARS in gastrocnemius muscles, alongside increased serum TC and TG (Fig. 1), supporting a link between systemic lipid overload and skeletal muscle oxidative stress. All three *G. frondosa* preparations attenuated TBARS, indicating protection against lipid peroxidation via the thiol-based antioxidants. The increases in GSH and/or GSH/GSSG ratios in MGF and MEP suggest maintenance of glutathione availability via recycling, enabling continued defense against oxidative stress. Furthermore, MGF's strong lipid-lowering effect (Fig. 1) may indirectly contribute to reduced oxidative stress in muscle by improving systemic lipid homeostasis.

# CONCLUSION

In conclusion, this study demonstrated that a HFHC diet induced dyslipidemia and lipid peroxidation across multiple non-adipose tissues. Fermented *G. frondosa* mycelium (MGF) and its intracellular (MIP) and extracellular (MEP) polysaccharides alleviated diet-induced hyperlipidemia and modulated oxidative stress in a tissue-specific manner. All three preparations reduced TBARS levels in the hearts and gastrocnemius muscles and increased GPx activity in the lungs, with MEP showing the most pronounced enhancement of total thiol content in lung tissues. Distinct effects included MGF lowering cardiac GSH and increasing muscle GSH, MIP lowering cardiac SOD activity and muscle total thiol content, and MEP improving lung redox balance while elevating renal TBARS. These findings support the potential of fermented *G. frondosa* mycelium and its polysaccharides as dietary strategies for lipid control and tissue-specific antioxidant protection.

## STUDY LIMITATION

The present study has several limitations. We did not analyze the regulatory effects of *G. frondosa* mycelia and its polysaccharides on genes or proteins involved in lipid metabolism and antioxidant pathways. In addition, further studies should examine the long-term impact of *G. frondosa* polysaccharides on systemic and local antioxidant capacity to provide a more comprehensive understanding of their molecular mechanisms. Moreover, the high-fat, high-cholesterol diet used in this study consisted solely of plant-derived corn and coconut oil and contributed 32% of total energy for eight weeks. Incorporating animal-based fats, increasing the proportion of dietary energy from fat, and extending the feeding duration may more clearly accentuate hyperlipidemia-related oxidative stress and tissue-specific responses.

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## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

## REFERENCES

1. Karr S. Epidemiology and management of hyperlipidemia. Am J Manag Care. 2017; 23: S139-48.
2. Zhang Z, Wu H, Wang T, Liu Y, Meng C. Mechanisms of myocardial damage due to hyperlipidemia: a review of recent studies. Med Sci Monit. 2022; 28: e937051.
3. van Herpen NA, Schrauwen-Hinderling VB. Lipid accumulation in non-adipose tissue and lipotoxicity. Physiol Behav. 2008; 94: 231-41.
4. Fiorenza M, Onslev J, Henríquez-Olguín C, et al. Reducing the mitochondrial oxidative burden alleviates lipid-induced muscle insulin resistance in humans. Sci Adv. 2024; 10: eadq4461.
5. Nie K, Deng T, Bai Y, et al. Association between composite dietary antioxidant index and hyperlipidemia in adults based on the NHANES. Sci Rep. 2025; 15: 2382.
6. Lee BH, Chen CH, Hsu YY, Chuang PT, Shih MK, Hsu WH. Polysaccharides obtained from *Cordyceps militaris* alleviate hyperglycemia by regulating gut microbiota in mice fed a high-fat/sucrose diet. Foods. 2021; 10: 1870.
7. Wang W, Zhang Y, Wang Z, Zhang J, Jia L. *Ganoderma lucidum* polysaccharides improve lipid metabolism against high-fat diet-induced dyslipidemia. J Ethnopharmacol. 2023; 309: 116321.
8. Wu JY, Siu KC, Geng P. Bioactive ingredients and medicinal values of *Grifola frondosa* (Maitake). Foods. 2021; 10: 95.
9. Wu WT, Hsu TH, Chen WL, Yang CK, Lo HC. Polysaccharides of *Grifola frondosa* ameliorate oxidative stress and hypercholesterolaemia in hamsters fed a high-fat, high-cholesterol diet. J Pharm Pharmacol. 2022; 74: 1296-306.
10. Wang YC, Lin FY, Hsu TH. Effects of nitrogen from different sources on mycelial biomass and polysaccharide production and pellet morphology in submerged cultures of *Grifola frondosa*. BioResources. 2021; 16: 2937-52.
11. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968; 25: 192-205.
12. Hissin PJ, Hilf R. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal Biochem. 1976; 74: 214-26.
13. Jiang X, Hao J, Liu Z, et al. Anti-obesity effects of *Grifola frondosa* through the modulation of lipid metabolism via ceramide in mice fed a high-fat diet. Food Funct. 2021; 12: 6725-39.
14. Yang M, Hu D, Cui Z, Li H, Man C, Jiang Y. Lipid-lowering effects of *Inonotus obliquus* polysaccharide in vivo and in vitro. Foods. 2021; 10: 3085.
15. Ge Y, Qiu H, Zheng J. Physicochemical characteristics and anti-hyperlipidemic effect of polysaccharide from Ba-Chu mushroom (*Helvella leucopus*). Food Chem X. 2022; 15: 100443.
16. Li L, Guo WL, Zhang W, et al. *Grifola frondosa* polysaccharides ameliorate lipid metabolic disorders and gut microbiota dysbiosis in high-fat diet fed rats. Food Funct. 2019; 10: 2560-72.
17. Newman PP, Schmitt BL, Maurmann RM, Pence BD. Polysaccharides with arabinose: key players in reducing chronic inflammation and enhancing immune health in aging. Molecules. 2025; 30: 1178.
18. Song YB, Kim B, Choi MJ, Song O, Cho EJ. Protective effect of arabinose and sugar beet pulp against high glucose-induced oxidative stress in LLC-PK1 cells. Food Chem. 2012; 134: 189-94.

19. Isago H. The association between dyslipidemia and pulmonary diseases. J Atheroscler Thromb. 2024; 31: 1249-59.
20. Tan M, Yin Y, Ma X, et al. Glutathione system enhancement for cardiac protection: pharmacological options against oxidative stress and ferroptosis. Cell Death Dis. 2023; 14: 131.
21. Noeman SA, Hamooda HE, Baalash AA. Biochemical study of oxidative stress markers in the liver, kidney and heart of high fat diet induced obesity in rats. Diabetol Metab Syndr. 2011; 3: 17.
22. Tramontano D, D'Erasmo L, Larouche M, et al. The vicious circle of chronic kidney disease and hypertriglyceridemia: What is first, the hen or the egg? Atherosclerosis. 2025; 403: 119146.
23. Gianazza E, Brioschi M, Fernandez AM, Banfi C. Lipoxidation in cardiovascular diseases. Redox Biol. 2019; 23: 101119.
24. Valaei K, Taherkhani S, Arazi H, Suzuki K. Cardiac oxidative stress and the therapeutic approaches to the intake of antioxidant supplements and physical activity. Nutrients. 2021; 13: 3483.
25. Skrzep-Poloczek B, Poloczek J, Che mecka E, et al. The oxidative stress markers in the erythrocytes and heart muscle of obese rats: relate to a high-fat diet but not to DJOS bariatric surgery. Antioxidants (Basel). 2020; 9: 183
26. Xiang Y, Xu X, Li J. Chemical properties and antioxidant activity of exopolysaccharides fractions from mycelial culture of *Inonotus obliquus* in a ground corn stover medium. Food Chem. 2012; 134: 1899-905.
27. Lash LH. Role of glutathione transport processes in kidney function. Toxicol Appl Pharmacol. 2005; 204: 329-42.
28. Turner N, Bruce CR, Beale SM, et al. Excess lipid availability increases mitochondrial fatty acid oxidative capacity in muscle: evidence against a role for reduced fatty acid oxidation in lipid-induced insulin resistance in rodents. Diabetes. 2007; 56: 2085-92.
29. Ingram KH, Hill H, Moellering DR, et al. Skeletal muscle lipid peroxidation and insulin resistance in humans. J Clin Endocrinol Metab. 2012; 97: E1182-6.
30. Pillon NJ, Croze ML, Vella RE, Soulère L, Lagarde M, Soulage CO. The lipidperoxidation by-product 4-hydroxy-2-nonenal (4-HNE) induces insulin resistance in skeletal muscle through both carbonyl and oxidative stress. Endocrinology. 2012; 153: 2099-111.

# 舞茸菌絲體及多醣體對餵食高脂肪高膽固醇飼料倉鼠高脂血症和組織氧化壓力的影響

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**摘要** 先前的研究證實，舞茸子實體可改善肝臟膽固醇代謝和高膽固醇血症。本研究的目的是探討舞茸液態發酵菌絲體（fermented mycelia of *Grifola frondosa*, MGF）及發酵液胞內（intracellular polysaccharides, MIP）與胞外（extracellular polysaccharides, MEP）多醣體對餵食高脂高膽固醇（HFHC）飲食倉鼠之非脂肪組織氧化壓力的影響。雄性倉鼠給予 HFHC 飲食，並管餵纖維素、MGF、MIP 或 MEP 八週，控制組接受一般飼料且管餵纖維素。結果顯示，MGF 與 MIP 顯著降低血清三酸甘油酯與膽固醇濃度，MEP 則降低膽固醇濃度。MGF、MIP 和 MEP 顯著降低心臟脂質過氧化物（TBARS）含量，然 MGF 和 MIP 分別減少還原型 glutathione（GSH）含量和 superoxide dismutase 活性。三種舞茸製備物均顯著提高肺臟 glutathione peroxidase 活性，且 MEP 同時降低 TBRAS 及增加總含硫化合物（total thiols）含量。然而，MEP 顯著增加腎臟 TBARS 含量。三種舞茸製備物皆顯著降低腓腸肌 TBARS 含量，MGF 和 MIP 降低總含硫化合物含量，但 MGF 增加 GSH 含量。綜上所述，MGF、MIP 與 MEP 可緩解 HFHC 引起的血脂異常，並提供組織特異性的抗氧化保護。此結果顯示，液態發酵舞茸菌絲體及其多醣體在預防高脂血症相關組織氧化損傷具有潛力。

**關鍵詞：舞茸、高血脂、多醣體、氧化壓力、抗氧化酵素**

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