# The anti-fatigue activities of *Tuber melanosporum* in a mouse model

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**Abstract.** *Tuber melanosporum* (TM) is an edible fungus that exhibits antioxidant and anti-tumor activity via its unique bioactive metabolites. The present study analyzed the anti-fatigue effects of TM using a BALB/c mouse model. The anti-fatigue properties of TM were evaluated by assessing the endurance of mice by performing forced swimming, rotary rod and running tests. Following 2 weeks TM treatment, hepatic and muscular ATP, and glycogen levels were increased in mice subjected to 30 min swimming, compared with controls. Similarly, levels of serum lactic acid and lactic dehydrogenase were decreased in the same group, compared with the control. Additionally, TM treatment reduced reactive oxygen species and malondialdehyde levels, and increased superoxide dismutase and glutathione peroxidase levels in the muscle, liver and/or serum. The effect of TM on hormone levels was also investigated in the present study, as different efficacies of TM were observed in male and female mice. TM treatment increased serum levels of progesterone, estradiol and testosterone in female and male mice, whereas a decrease in serum luteinizing hormone levels was only observed in females. A decrease in serum follicle-stimulating hormone levels was identified in females, whereas an increase was observed in males. The current study demonstrated that the anti-fatigue effects of TM occur via the regulation of oxidative stress, energy metabolism and hormone levels.

## Introduction

Fatigue is defined as a difficulty in the initiation or sustainment of voluntary activity and is caused by serious stress and/or intensive physical or mental activity (1). Fatigue can therefore be divided into two categories: Physical and mental fatigue. Physical fatigue is the inability of a muscle to maintain normal movement (2), which may induce a heightened stress response, endocrine dyscrasia, immune dysfunction and organ injury (3).

It has been demonstrated that metabolic dysfunction, oxidative stress and lipid peroxidation resulting from excessive exercise can lead to physical fatigue (4). Additionally, the accumulation of metabolic by-products, such as lactic acid (LA) and the depletion of energy resources, including adenosine triphosphate (ATP) and glycogen, may cause metabolic dysfunction (5). The accumulation of harmful metabolites is eliminated and the repair of damaged cells occurs following the consumption of proteins, saccharides and fatty acids post-exercise (6). However, an imbalance between levels of reactive oxygen species (ROS) and antioxidants may induce oxidative stress (7), leading to lipid peroxidation (8). Malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels reflect antioxidant capacity (9,10). It has been demonstrated that reversing the depletion of energy resources and inhibiting the generation of free radicals induces positive effects on anti-fatigue and physical abilities, respectively (11).

Due to the high prevalence of fatigue, screening for agents that may prevent it and ensure a rapid recovery has become an attractive prospect (12). Various species of fungi and certain herbs may postpone the onset of fatigue, improve athletic ability and enhance the elimination of fatigue-related metabolites (13). The anti-fatigue effects of *Tricholoma matsutake, Cordyceps militaris* and *Antrodia cinnamomea* in murine models have been examined in previous studies by assessing their effect on oxidative stress (3,14,15). *Tuber melanosporum* (TM), a fungus containing unique bioactive metabolites, contains important nutrients including proteins, unsaturated fatty acids, amino acids, sphingolipids, cerebrosides and polysaccharides (16). It has been demonstrated that TM exhibits antioxidant and anti-tumor activity in A549, HepG2 and HL-60 cells (17). The anti-fatigue effects of TM have not yet been examined; thus it was hypothesized that its function as an antioxidant may prevent the symptoms of fatigue. Therefore, the present study assessed the anti-fatigue effects of TM using a BALB/c mouse model. Energy metabolism, the balance

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between pro- and antioxidants and hormone levels in the mice were assessed to reveal the mechanisms by which TM may improve endurance during exercise.

## Materials and methods

*TM preparation*. TM fungus was purchased from Senzhong Co., Ltd. (Yunnan, China) and broken apart using a crushing machine. Preliminary experiments identified the constituent components of TM using the phenol-sulfate method (18), bicinchoninic acid protein assay (19), rutin standard colorimetry (20) and high performance liquid chromatography (21). The amount of polysaccharides, proteins, flavone and adenosine in TM were determined to be 10.8, 14.2, 0.4 and 0.1%, respectively.

*Animals*. A total of 96 BALB/c mice (age, 8 weeks; weight, 20-22 g; sex ratio, 48 male and 48 female) were purchased from the Changchun Institute of Biological Products Co., Ltd. (Jilin, China) and were maintained under a 12 h light/dark cycle (lights on between 7:00 a.m. and 7:00 p.m.) at 23±1˚C with 50±5% humidity. Food and water was available *ad libitum*. All experiments were performed in a quiet room. The experimental animal protocol used in the present study was approved by the Animal Ethics Committee of Jilin University (Jilin, China; approval no. 2017 nsfc0005).

*Measurement of anti-fatigue activity*. Following 1 week adaptation, mice were randomly divided into 4 equal groups (n=24; consisting of equal numbers of males and females). Mice underwent treatment for a total of 21 days and were either orally treated with physiological saline (control, 0.01 ml/g) or TM at doses of 0.25, 0.5 or 1.0 g/kg once a day. Doses and administration routes of TM were selected based on previous studies (14). The experimental protocol and drug administrations are presented in Fig. 1.

*Independent activity test*. On day 15 of treatment, 30 min following TM administration, the mice were placed in an autonomous chamber (ZZ-6; Chengdu Techman Software Co., Ltd., Chengdu, China). Following 2 min adaption time the number of horizontal movements and vertical movements made by the mice were recorded for 5 min. The test was repeated three times.

*Forced running test*. On day 16 of treatment, 30 min following TM administration, mice were placed on a treadmill (FT-200; Chengdu Techman Software Co., Ltd.) to assess their endurance capabilities. Following three preliminary test sessions at a speed of 20 rpm/min for 1 min, the formal test was performed at the same speed and the time period until the mice fell off due to muscle fatigue was measured. Exhaustion time was identified as failure to run for 10 sec following an electric shock (1 mA), which motivates the mice to run, as previously described (22).

*Rotating rod test*. On day 18 of treatment, 30 min following TM administration, mice were placed on a turning device (ZB-200, Chengdu Techman Software Co., Ltd.) and performed rotating rod training exercises at a speed of 15 rpm for 1 min, which were repeated three times. During the formal experiment, the speed was set up to 20 rpm and the time taken for mice to succumb to muscle fatigue and fall off the equipment was recorded (3).

*Weight loaded forced swimming test*. On day 19 of treatment, 30 min following TM administration, weights weighing 5% of the mouse's total body weight were added to each mouse, which were placed into water at a temperature and depth of 22±1˚C and 30 cm, respectively. Exhaustion time was identified as the failure of mice to return to the water's surface within 8 sec (23).

*Sample collection and parameter determination*. On day 21, mice were forced to swim in water at a temperature and depth of 22±1˚C and 30 cm respectively, for 30 min. Following a 10 min recess, blood samples were collected from the caudal veins and the mice were subsequently sacrificed. Following 10 min at room temperature the blood samples were centrifuged at 604 x g for 15 min at room temperature and the upper layer of serum was collected. Liver and muscle tissue was quickly dissected, washed in ice-cold physiological saline solution and homogenized in double distilled water.

The serum levels of LA (cat. no. CK-E93905), lactic dehydrogenase (LDH; cat. no. CK-E20034), progesterone (PROG; cat. no. CK-E20376), follicle stimulating hormone (FSH; cat. no. CK-E20419), estradiol ($E_2$; cat. no. CK-E20381), testosterone (T; cat. no. CK-E20375) and luteinizing hormone (LH; cat. no. CK-E20343), alongside the serum, liver and muscular levels of SOD (cat. no. CK-E20348), GSH-Px (cat. no. CK-E92669) and MDA (cat. no. CK-E20347) were detected using ELISA according to the protocols provided by the relevant assay kits (Shanghai Yuanye Bio-Technology Co. Ltd, Shanghai, China). ATP levels (cat. no. CK-E93365) in the liver and muscles of mice was also assessed via ELISA. The concentration of muscle glycogen (MG) and hepatic glycogen (HG; cat. no. A043) as well as ROS levels (cat. no. E004) in the liver and muscles of mice were detected using the relevant assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's protocol.

*Statistical analysis*. All data were expressed as the mean ± standard error of the mean. One-way analysis of variance followed by Dunn's multiple comparisons was used to evaluate differences among groups. Data were analyzed using SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA) and $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*The anti-fatigue effects of TM*. TM administration did not significantly affect the body weights of mice (Table I). In the rotating rod test, TM at 0.25 g/kg caused a 67% increase in the rotating time in female mice and TM at 1.0 g/kg caused a 117% increase in the rotating time in male mice, which were each significantly increased compared with the control group ($P<0.05$; Fig. 2A). In the forced running test, no significant increase in the exercise times of male mice

Table I. Effects of TM on the weights of female and male mice.

| Group | TM dose (g/kg) | 14-day drug treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 | 12 | 14 |
| Female | | | | | | | | | |
| CTRL | 0 | 20.2±0.3 | 20.2±0.4 | 19.9±0.3 | 20.1±0.3 | 20.2±0.3 | 20.3±0.3 | 19.9±0.3 | 20.3±0.3 |
| TM 1 | 0.25 | 19.5±0.5 | 20.1±0.4 | 20.2±0.4 | 20.0±0.4 | 19.5±0.4 | 20.3±0.4 | 19.9±0.5 | 20.1±0.4 |
| TM 2 | 0.5 | 20.3±0.5 | 19.7±0.5 | 20.6±0.4 | 20.3±0.4 | 19.9±0.4 | 20.0±0.5 | 19.9±0.5 | 20.2±0.5 |
| TM 3 | 1.0 | 20.5±0.3 | 20.7±0.4 | 20.4±0.4 | 20.3±0.4 | 20.3±0.4 | 20.7±0.4 | 20.3±0.3 | 20.6±0.4 |
| Male | | | | | | | | | |
| CTRL | 0 | 20.7±0.5 | 20.1±0.5 | 20.2±0.4 | 20.3±0.4 | 20.2±0.3 | 20.3±0.3 | 20.4±0.4 | 20.7±0.4 |
| TM 1 | 0.25 | 20.6±0.5 | 19.3±0.5 | 19.8±0.5 | 19.1±0.5 | 19.3±0.6 | 19.6±0.5 | 19.4±0.5 | 19.9±0.5 |
| TM 2 | 0.5 | 20.0±0.5 | 19.6±0.5 | 20.3±0.5 | 20.1±0.4 | 20.1±0.4 | 19.7±0.6 | 19.8±0.4 | 20.1±0.5 |
| TM 3 | 1.0 | 20.7±0.5 | 20.6±0.4 | 20.8±0.3 | 20.3±0.3 | 20.1±0.4 | 20.6±0.3 | 20.3±0.4 | 20.7±0.3 |

Mice were treated with TM doses of 0.25, 0.5 or 1.0 g/kg for 2 weeks. Body weights of female and male mice were recorded. Values are presented as mean ± standard error of the mean (n=12). TM, *Tuber melanosporum*; CTRL, control.

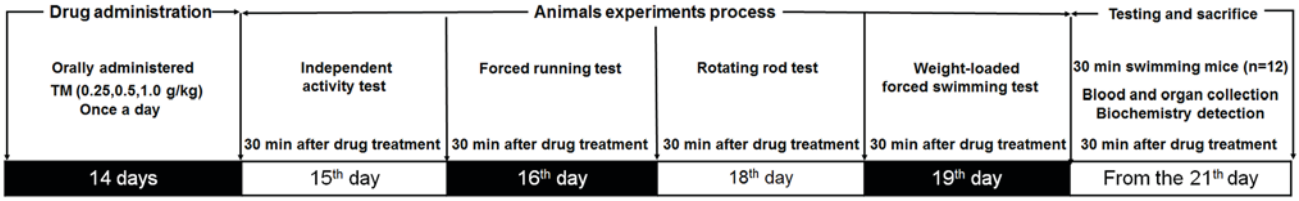


Figure 1. Experimental protocol and drug administration. TM, *Tuber melanosporum*.

was observed following TM treatment. In contrast, TM at a dose of 0.25 and 1.0 g/kg resulted in a 40 and 78% increase in the running times of female mice, which was significantly increased compared with the control group (P<0.01; Fig. 2B). Treatment with 0.25 g/kg TM improved the swimming times of female and male mice by 51 and 58%, respectively, which were significantly increased compared with the control group (P<0.05 and P<0.01; Fig. 2C). Additionally, treatment with TM did not influence the horizontal and vertical movements of female or male mice included in the independent activity tests (data not shown).

*The antioxidant effects of TM in mice following 30 min exercise*. It has been demonstrated that the overproduction of ROS *in vivo* causes oxidative damage and deleterious effects (7). Additionally, excessive quantities of MDA lead to free radical generation and lipid peroxidation, which may dysregulate cell function and induce fatigue (9). Antioxidant enzymes, including GSH-Px and SOD, serve important roles in preventing excessive exercise-induced oxidative injury in animals (10). Following 30 min swimming exercise, female mice treated with TM exhibited reduced serum MDA levels compared with the control. Male and female mice treated with 0.25 g/kg TM exhibited significantly decreased serum MDA compared with the control group (P<0.05 and P<0.01; Table II); Treatment with 0.5 g/kg TM significantly increased the serum levels of SOD and GSH-Px in male mice (P<0.01 and P<0.001; Table II), but not female mice (Table II).

The effect of TM treatment on levels of oxidation factors in the livers of female and male mice following 30 min exercise was assessed. In female mice, 1.0 g/kg TM significantly reduced MDA levels in the serum of the liver by 15% compared with the control mice (P<0.05; Table II). All doses of TM exhibited significantly reductive effects on ROS levels in the liver, particularly at 1.0 g/kg, which resulted in >60% reduction (P<0.05; Table II). Treatment with 0.25 g/kg TM significantly enhanced SOD and GSH-Px levels in female mice in the liver (P<0.05 and P<0.01; Table II). Conversely, in male mice TM 0.25 g/kg demonstrated no significant effects on the levels of SOD and GSH-Px in the liver (Table II). Treatment with 0.25 g/kg TM in male mice caused significantly reduced hepatic MDA and ROS levels (P<0.01; Table II) compared with the control.

The effect of TM treatment on levels of oxidation factors in the muscle of male and female mice 30 min after administration was also examined. Following TM treatment, levels of MDA (1.0 g/kg TM) and ROS (0.25 and 1.0 g/kg TM) were significantly reduced in the muscle of female mice compared with control (P<0.001 and P<0.01; Table II) SOD and GSH-Px were unaffected in the muscles of female mice following treatment with all doses of TM. However, male mice treated with TM, at 1.0 g/kg, exhibited significantly decreased muscular MDA (P<0.05) and ROS (P<0.01) and

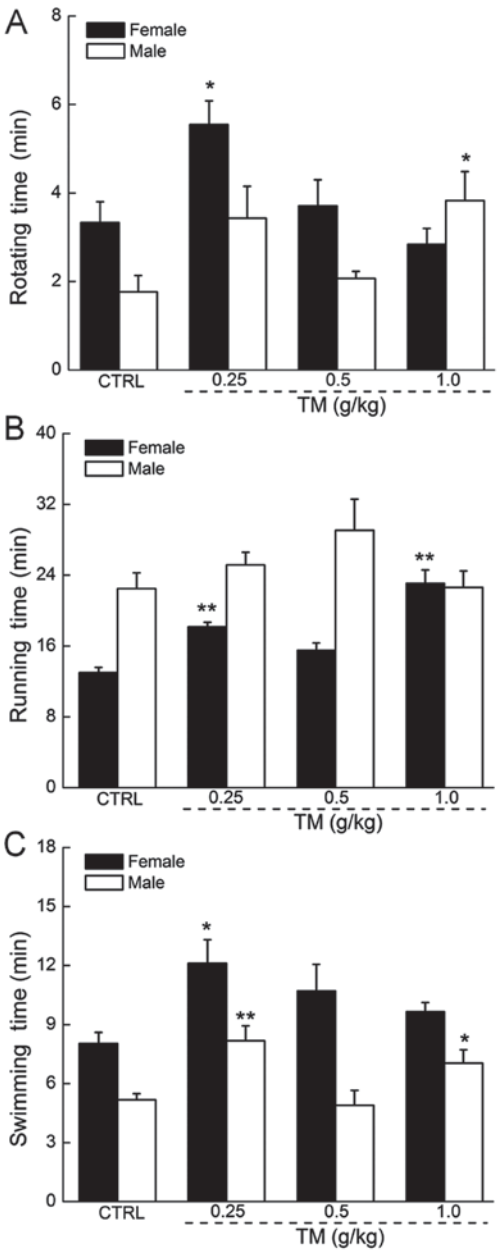


Figure 2. Administration with TM over 2 weeks altered endurance times in male and female mice during (A) forced running, rotating rod (B) and (C) forced swimming tests. Data are expressed as mean ± standard error of the mean. (n=12) and analyzed using one-way analysis of variance followed by a post-hoc Dunn's test. *P<0.05 and **P<0.01 vs. CTRL. TM, *Tuber melanosporum*; CTRL, control group.

significantly increased SOD and GSH-Px levels, compared with the control (P<0.05; Table II).

*The regulatory effects of TM on ATP and glycogen in the muscle and liver tissue of mice*. The absence of ATP in hepatic and muscular tissue may cause membrane damage (3). Following 30 min forced swimming, TM dose-dependently, enhanced the muscular ATP levels in male mice, which were all significantly increased compared with the control (P<0.05 and P<0.01; Fig. 3A). In contrast, only TM at 1.0 g/kg significantly increased the muscular ATP levels in female mice (P<0.05; Fig. 3A). Compared with the controls, 0.25 g/kg TM caused a significant 19 and 33% increase in hepatic ATP levels in female and male mice, respectively (P<0.05; Fig. 3B).

Glycogen is the predominant source of energy during exercise, and its depletion may be a primary element for the development of physical fatigue (24). A shortage of hepatic glycogen may severely impair nervous function (25). Male and female mice treated with TM following 30 min forced swimming exhibited significantly increased MG concentration in all doses, compared with the control (P<0.05 and P<0.01; Fig. 3C). TM administration did not significantly affect hepatic glycogen levels in male and female mice. However, female mice treated with TM exhibited increased hepatic glycogen concentration of ~20% compared with controls (P<0.05; Fig. 3D).

*The regulatory effects of TM on LA and LDH levels in the serum*. In anaerobic conditions, LA accumulates and affects the ability of an individual to maintain intense exercise (26). Only female mice treated with 1.0 g/kg TM exhibited significantly reduced LA serum levels compared with the control (3.5 mmol/l vs. 4.1 mmol/l; P<0.05; Fig. 4A). Other doses of TM did not significantly affect LA serum levels in females. LA serum levels in males remained unchanged following treatment with all doses of TM. In addition, excessive quantities of LDH serve as an indicator of muscle damage (27). Following 30 min exercise, a 14-day treatment of 1.0 g/kg TM reduced serum LDH levels by 15.9 and 11.4% in female and male mice respectively compared with the control (both P<0.05; Fig. 4B). Other doses of TM did not significantly affect LDH levels in male or female mice.

*The effects of TM treatment on serum hormone concentrations*. As TM exhibited different efficacies in male and female mice, its effects on hormone levels were evaluated. Treatment with 0.25 g/kg TM significantly increased serum PROG, E2 and T in female mice compared with the control group (P<0.05 and P<0.01; Table III). Treatment with 0.25 g/kg TM significantly increased serum T (P<0.01) and FSH (P<0.05; Table III) and 1.0 g/kg TM significantly increased serum PROG, FSH and E2 (P<0.05; Table III) in male mice compared with the control group. Female mice treated with 0.5 g/kg of TM exhibited a 12.2% decrease in serum FSH, while male mice undergoing the same dose treatment exhibited an increase of 33% (P<0.05; Table III). Additionally, female mice treated with 1.0 g/kg of TM exhibited a decrease of 20% of serum LH (P<0.05; Table III), however, TM treatment did not significantly affect serum LH levels in male mice.

## Discussion

Excessive energy consumption and the accumulation of metabolic products as a result of physical and mental stress may cause irreversible oxidative tissue damage and lead to depressed immunity, accelerated ageing and cardio-cerebrovascular disease (28,29). The present study identified the anti-fatigue properties of TM in a mouse model by performing forced swimming, rotary rod and exhausted running tests, which are considered to be valid methods of evaluating the exercise capacity of mice (30). It was demonstrated that the same dose of TM had different effects in different tests, and TM was observed to have a non-dose dependent effect in the majority

Table II. Effects of TM on oxidative stress-related factors in the serum, liver and muscle of female and male mice.

| | Female | | | | Male | | | |
|---|---|---|---|---|---|---|---|---|
| | | TM (g/kg) | | | | TM (g/kg) | | |
| Factor | CTRL | 0.25 | 0.5 | 1.0 | CTRL | 0.25 | 0.5 | 1.0 |
| Serum | | | | | | | | |
| MDA (nmol/ml) | 13.2±0.3 | 11.8±0.2[a] | 10.8±0.2[b] | 11.6±0.4[a] | 11.0±0.3 | 9.6±0.2[b] | 11.5±0.1 | 10.7±0.1 |
| SOD (U/ml) | 126.8±4.8 | 129.5±7.8 | 123.9±1.2 | 108.6±3.1 | 156.1±6.3 | 163.5±1.8 | 190.5±6.4[b] | 191.8±6.5[b] |
| GSH-Px (μmol/ml) | 87.5±10.6 | 78.3±6.8 | 75.5±10.2 | 74.2±4.9 | 375.2±4.4 | 374.1±5.9 | 409.7±1.0[c] | 383.9±3.8 |
| Liver | | | | | | | | |
| MDA (nmol/mgprot) | 3.9±0.1 | 4.2±0.1 | 4.1±0.2 | 3.3±0.1[a] | 3.7±0.1 | 2.8±0.2[b] | 3.5±0.1 | 3.9±0.2 |
| ROS (FI/mgprot) | 21,847±2,465 | 11,175±2,076[a] | 14,179±912[a] | 8,758±1,146[b] | 31,630±1,316 | 21,810±1,072[b] | 8,523±2,562[c] | 10,976±972[c] |
| SOD (U/mgprot) | 36.8±2.1 | 47.1±1.8[a] | 41.1±1.4 | 44.1±3.7 | 39.4±0.9 | 40.9±1.1 | 40.1±0.9 | 36.3±1.2 |
| GSH-Px (μmol/gprot) | 54.2±2.9 | 74.1±3.8[b] | 80.4±2.7[c] | 51.2±1.4 | 45.6±1.3 | 43.1±2.1 | 44.3±1.8 | 47.8±1.4 |
| Muscle | | | | | | | | |
| MDA (nmol/mgprot) | 7.6±0.1 | 7.1±0.5 | 6.8±0.5 | 2.8±0.1[c] | 10.2±0.8 | 6.6±0.5[a] | 7.8±0.4 | 7.2±0.7a |
| ROS (FI/gprot) | 444,351±11,198 | 346,146±3,3050[a] | 417,217±12,991 | 196,190±56,406[b] | 544,315±33,451 | 402,723±2,9494[a] | 418,932±4,461 | 324,673±11,594[b] |
| SOD (U/mgprot) | 61.7±1.6 | 58.0±3.2 | 57.9±2.6 | 58.9±5.9 | 64.6±2.9 | 68.5±5.3 | 80.5±2.6[a] | 74.6±6.3[a] |
| GSH-Px (μmol/gprot) | 68.9±2.6 | 74.7±2.6 | 71.6±3.8 | 74.9±4.6 | 82.9±5.6 | 98.0±4.8[a] | 123.5±2.8[b] | 108.3±5.8[a] |

Mice were treated with TM at doses of 0.25, 0.5 and 1.0 g/kg for 2 weeks. Levels of MDA, ROS, SOD and GSH-Px in the liver, muscle and/or serum of female and male mice were detected using ELISA. Values are expressed as mean ± standard error of the mean (n=12). [a]P<0.05, [b]P<0.01 and [c]P<0.001 vs. CTRL. TM, *Tuber melanosporum*; CTRL, control; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase.

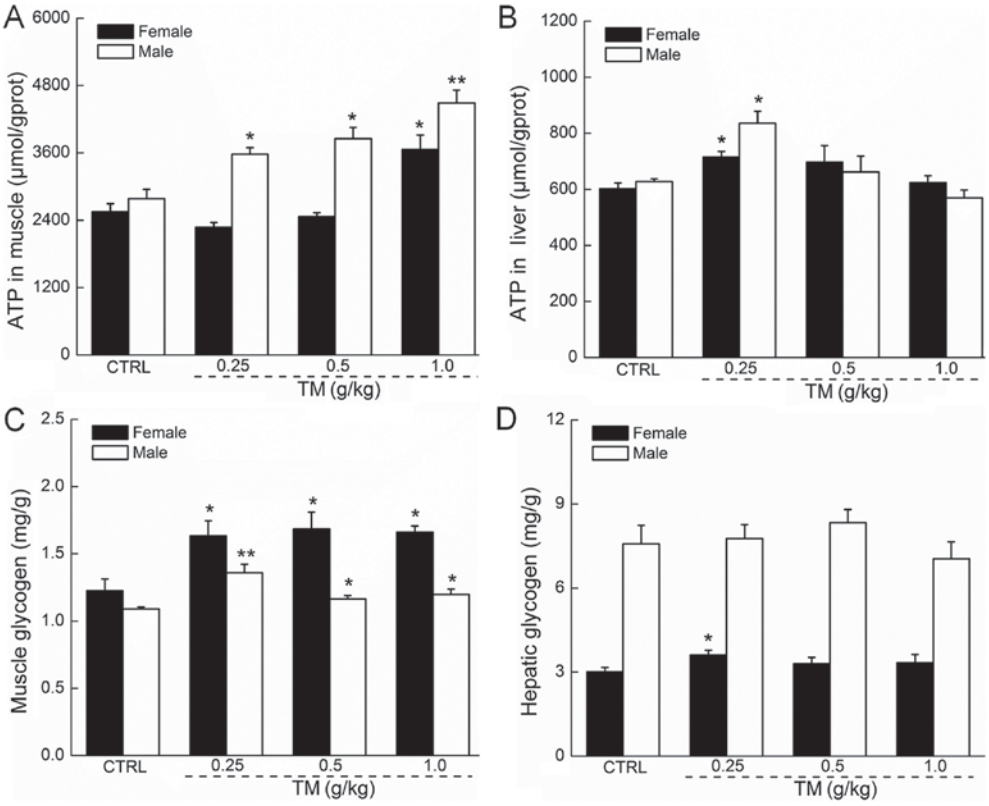


Figure 3. Administration with TM over 2 weeks changed the levels of ATP in (A) muscle and (B) liver tissue, and affected the levels of (C) MG and (D) HG in male and female mice. Data are expressed as mean ± standard error of the mean (n=12) and analyzed using one-way analysis of variance followed by a post-hoc Dunn's test. $^{*}$P<0.05 and $^{**}$P<0.01 vs. CTRL. TM, *Tuber melanosporum*; ATP, adenosine triphosphate; MG, muscle glycogen; HG, hepatic glycogen; CTRL, control group.

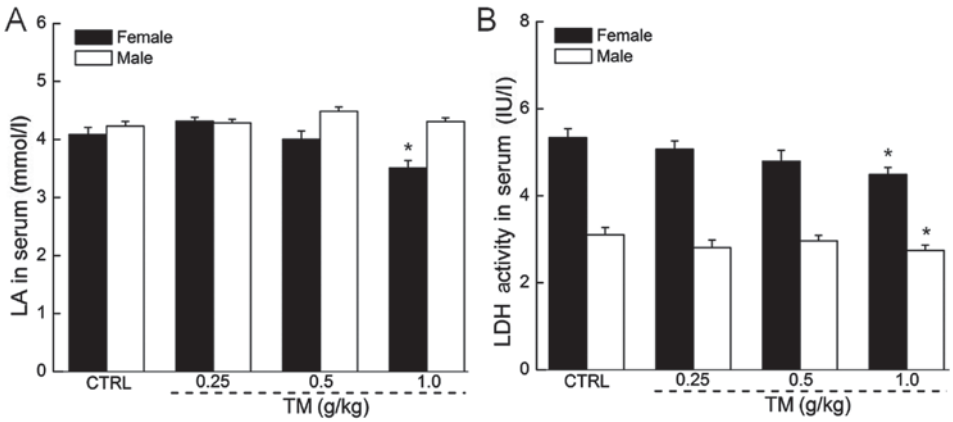


Figure 4. Administration with TM over 2 weeks affected the serum levels of (A) LA and (B) LDH in male and female mice Data are expressed as mean ± standard error of the mean (n=12) and analyzed using one-way analysis of variance followed by a post-hoc Dunn's test. $^{*}$P<0.05 vs. CTRL. TM, *Tuber melanosporum*; LA, lactic acid; LDH, lactic dehydrogenase; CTRL, control group.

of the experiments. TM contains multi-effective components, which may demonstrate a synergistic effect on exercise tolerance. This may explain the non-dose dependent effects of TM.

ATP, the primary rapid and direct energy source for exercise, is affected by the activities of myofibrillar ATPase (31). In the present study, following TM administration and 30 min subsequent swimming exercise, 0.25 g/kg TM significantly increased hepatic ATP and 1.0 g/kg TM significantly increased muscular ATP in male and female mice. Glycogen is regarded as an immediate source of energy for the synthesis of ATP, which is important as ATP possesses an ultra-short half-life (3). The quantity of glycogen within tissues may serve as an indicator for persistent exercise-induced fatigue, as a reduction of glycogen is observed in these conditions (32). Following 30 min forced swimming, mice treated with TM exhibited increased levels of muscular glycogen compared with controls. Additionally LA, which is primarily produced during anaerobic glucose metabolism, reduces the pH of blood and muscle, inducing exercise-induced fatigue and

Table III. Effects of TM on serum hormone levels of female and male mice.

| | Female | | | | Male | | | |
|---|---|---|---|---|---|---|---|---|
| | | TM (g/kg) | | | | TM (g/kg) | | |
| Hormone | CTRL | 0.25 | 0.5 | 1.0 | CTRL | 0.25 | 0.5 | 1.0 |
| PROG (ng/ml) | 3.2±0.03 | 4.1±0.2[a] | 3.4±0.1 | 2.9±0.1 | 9.1±0.2 | 10.1±0.3 | 10.4±0.3[a] | 10.7±0.3[a] |
| FSH (mIU/ml) | 37.8±1.5 | 35.6±1.1 | 33.2±1.2[a] | 34.8±0.7 | 18.1±0.9 | 21.5±0.5[a] | 24.1±1.3[a] | 22.9±0.7[a] |
| E2 (pmol/l) | 65.6±0.9 | 72.1±0.5[a] | 63.2±1.3 | 65.7±1.3 | 43.8±0.7 | 45.1±0.5 | 46.6±0.3 | 47.3±0.4[a] |
| T (pg/ml) | 108.5±9.0 | 154.7±3.6[b] | 128.6±9.6[a] | 104.3±6.2 | 469.6±11.1 | 531.1±7.7[b] | 500.4±65.4 | 445.6±18.1 |
| LH (mIU/ml) | 6.6±0.2 | 6.3±0.1 | 4.5±0.3[b] | 5.3±0.2[a] | 5.4±0.2 | 5.7±0.08 | 5.8±0.04 | 5.9±0.1 |

Mice were treated with TM at doses of 0.25, 0.5 and 1.0 g/kg for 2 weeks. The serum levels of PROG, FSH, E2, T and LH in female and male mice were detected using ELISA. Values are expressed as mean ± standard error of the mean (n=12). [a]P<0.05 and [b]P<0.01 vs. CTRL. TM, *Tuber melanosporum*; CTRL, control; PROG, progesterone; FSH, follicle-stimulating hormone; E2, estradiol; T, testosterone; LH, luteinizing hormone.

tissue damage (26). Under normal conditions, muscular LDH catalyzes the mutual transformation of LA and pyruvate (24). High serum LDH levels are also a marker of muscle damage (27). The present study demonstrated that TM possesses anti-fatigue properties, as 1.0 g/kg TM treated female mice exhibited significantly reduced serum LA and LDH levels.

Oxygen can be transformed into oxygen free radicals (OFR), which serve important roles in signal transduction and other physiological processes (14). Unremitting and strenuous exercise increases energy consumption and accelerates the accumulation of OFR derivatives, such as ROS, leading to oxidative stress and muscle fatigue. ROS may combine with macromolecules, including DNA and proteins, causing lipid peroxidation and muscle fatigue (33). MDA regulates ATP synthase via the mitochondrial respiratory chain, which is an indirect indicator of membrane damage (34). Enzymatic and non-enzymatic antioxidants exhibit two classic patterns of endogenous protective mechanisms to prevent oxidative stress (35). Antioxidant enzymes, including SOD and GSH-Px, may clear accumulated OFR and associated metabolites to maintain homeostasis and attenuate the effects of ROS, thus protecting cellular structures from destruction and prevent the onset of fatigue (36). In the present study, TM treated female and male mice exhibited increased serum, hepatic and muscular SOD and GSH-Px, and decreased MDA and ROS levels to different degrees depending on the concentration of TM administered. *Antrodia cinnamomea* and *Polygonatum alte-lobatum* hayata reduce MDA levels and increase the activity of antioxidant enzymes including SOD and GSH-Px, thus protecting cellular structures by combating lipid peroxidation and fatigue (10,15). The antioxidant properties of TM may therefore be responsible for its anti-fatigue effects.

In the present study, the serum hormone levels of mice were assessed, as TM treatment exhibited different degrees of anti-fatigue activity in males compared with females. The results indicated that TM regulates FSH and LH levels differently in male and female mice. It has been demonstrated that TM induces estrogen-like effects and increases the indices of the ovaries and uterus [ovaries or uterus indice=ovaries or uterus weight (mg)/body weight (g)] (37). Long-term and high-intensity exercise inhibits the secretion of gonadotropin-releasing hormone from the hypothalamus which, in turn, inhibits the secretion of LH and FSH from the pituitary (38). LH and FSH have synergistic effects on testicular stromal cells and may alleviate the dysfunction caused by the hypothalamus-pituitary-gonadal axis (38). However, the hypothalamic-pituitary-testicular axis can be stimulated by continuous exercise, resulting in T secretion, which increases exercise capacity (39). PROG and E2 also reduce muscular fatigue, which may increase performance of certain physical activities (40). Based on the aforementioned studies, TM-mediated hormone regulation may contribute to its anti-fatigue effects, which may also serve to explain the differences in its effects on male and female mice in the present study.

The present study did have limitations. TM exhibited different effects on excise performance in male and female mice. Although it was observed that TM effectively regulated hormone levels in male and female mice, the underlying mechanisms for how it does this are still unclear and this requires further investigation to explore in more detail.

In conclusion, the results of the present study indicate that the anti-fatigue effects observed in mice following TM treatment are primarily caused by the regulation of oxidative stress, energy metabolism and hormone levels. This suggests that TM may increase endurance capabilities during exercise, which provides experimental evidence to support the clinical use of TM as a functional natural product against fatigue.

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