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Increase in muscle endurance in mice by dietary Yamabushitake mushroom (*Hericium erinaceus*) possibly via activation of PPARδ

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Abstract

Skeletal muscle fiber is largely classified into two types: type 1 (slow-twitch) and type 2 (fast-twitch) fibers. Meat quality and composition of fiber types are thought to be closely related. Previous research showed that overexpression of constitutively active peroxisome proliferator-activated receptor (PPAR)δ, a nuclear receptor present in skeletal muscle, increased type 1 fibers in mice. In this study, we found that hexane extracts of Yamabushitake mushroom (*Hericium erinaceus*) showed PPARδ agonistic activity in vitro. Eight-week-old C57BL/6J mice were fed a diet supplemented with 5% (w/w) freeze-dried Yamabushitake mushroom for 24 hr. After the treatment period, the extensor digitorum longus (EDL) muscles were excised. The Yamabushitake-supplemented diet up-regulated the PPARδ target genes *Pdk4* and *Ucp3* in mouse skeletal muscles in vivo. Furthermore, feeding the Yamabushitake-supplemented diet to mice for 8 weeks resulted in a significant increase in muscle endurance. These results indicate that Yamabushitake mushroom contains PPARδ agonistic ligands and that dietary intake of Yamabushitake mushroom could activate PPARδ in skeletal muscle of mice. Unexpectedly, we observed no significant alterations in composition of muscle fiber types between the mice fed control and Yamabushitake-supplemented diets.

KEYWORDS

Hericium erinaceus, muscle endurance, muscle fiber type, mushroom, PPARδ

1 | INTRODUCTION

In the skeletal muscle of most animals, there are two major classifications of fiber type; namely, type 1 and type 2 fibers. Type 2 fibers are further subdivided into type 2A, 2X, and 2B fibers (Schiaffino & Reggiani, 2011). Type 2A and 2X fibers have characteristics intermediate between those of type 1 and type 2B fibers. Although type 2X fibers are sometimes recognized as fast-twitch glycolytic fibers, type 2B fibers have an even stronger fast-twitch glycolytic phenotype (Rivero, Talmadge, & Edgerton, 1999). These fiber types are generally classified according to myosin heavy chain (MyHC) isoforms. In rodent skeletal muscles, four

adult MyHC isoforms have been identified: MyHC1, 2A, 2X, and 2B, preferentially expressed respective fiber types. Within a muscle, the composition of muscle fiber types determines a variety of skeletal muscle properties, including contractile (slow-twitch or fast-twitch, fatigue-resistant or fatigable) and metabolic characteristics (oxidative or glycolytic).

Several studies suggest that muscle fiber-type composition is also related to meat quality. For example, a higher composition of fast-twitch-type 2B fibers in pork meat is positively correlated with meat lightness and drip loss (Ryu & Kim, 2005). In sensory evaluation studies, the ratio of fast-twitch to slow-twitch is negatively correlated with juiciness and flavor of Berkshire pigs, suggesting that an increase in fast-twitch fibers would reduce scores for juiciness and flavor (Kang et al., 2011). Furthermore, in tough pork meat with a higher shear force value, as assessed by the Warner–Bratzler method, the expressions of fast-type MyHC2X (MYH1) and myosin light chain 1 (MYL1) were up-regulated (Hamill et al., 2012). Moreover, type 1 fibers contain more lipids than do type 2B fibers (Alasnier, Remignon, & Gandemer, 1996). In muscle tissue mainly composed of type 2B fibers, levels of imidazole dipeptides, such as carnosine and anserine, are higher, whereas in muscles predominantly composed of type 1 fibers, levels of taurine and glutamine are higher (Aristoy & Toldra, 1998; Cornet & Bousset, 1999). Therefore, establishing a method to modify muscle fiber-type composition could help control meat quality.

The composition of muscle fiber type in adult skeletal muscles can change in response to hormonal status (particularly by thyroid hormones; (Izumo, Nadal-Ginard, & Mahdavi, 1986) or activation/contraction patterns; for example, by external electrical stimulation (Pette & Staron, 1997), paralysis due to spinal cord injury (Talmadge et al., 2004), denervation (Sato et al., 2009), cross-reinnervation (fast muscle reinnervated by a slow nerve and vice versa; (Salmons & Sreter, 1976), or mechanical unloading (Caiozzo, Baker, & Baldwin, 1997). In a recent study, we found that intake of certain food ingredients induced a transition in muscle fiber type in rat skeletal muscles. An 8-wk supplementation with 0.5% apple polyphenols (APP) significantly up-regulated slower MyHC isoforms in hind limb skeletal muscles of rats (Mizunoya et al., 2017), and supplementation with 5% APP increased skeletal muscle endurance capacity and altered muscle fiber type (increase of fibers expressing MyHC1; (Mizunoya et al., 2015). Furthermore, we reported that a transition from type 2B to type 2X fibers in extensor digitorum longus (EDL) muscles (MyHC2B was most abundant) was induced in rats fed fish oil compared with those fed soybean oil (Mizunoya et al., 2013). These reports suggest that dietary components have the potential to change skeletal muscle fiber type.

Skeletal muscle fiber-type composition is regulated by several transcription factors, of which one of the most important is peroxisome proliferator-activated receptor (PPAR)δ. Overexpression of a constitutively active PPARδ (VP16-PPARδ) in skeletal muscle resulted in a profound and coordinated increase in oxidative enzymes, mitochondrial biogenesis, and production of slow-twitch fibers (Wang et al., 2004). Luquet et al. (2003) reported that PPARδ expression in skeletal muscle is promoted by exercise training, which is the typical environmental stimulus leading to an increase of type 1 fibers. To date, few studies have shown that natural food components could activate PPARδ. For example, Cho et al. reported that an ethanol extract from Haninjin (*Artemisia iwayomogi*) had PPARδ agonistic activity and up-regulated mRNA expression of PPARδ target genes in an in vitro study (Cho et al., 2012). Other reports showed that compounds in the roots of Ku-Shen (*Sophora flavescens*) (Quang et al., 2013) and hydroxyeicosapentaenoic acids from the Pacific krill (*Euphausia pacifica*) (Yamada et al., 2014), had high agonistic activity for PPARδ.

To further evaluate dietary components that activate PPARδ, we focused on Yamabushitake mushroom (*Hericium erinaceus*), an edible mushroom popular in east Asia that is used as a traditional Chinese medicinal mushroom. The Yamabushitake mushroom has been shown to promote synthesis of nerve growth factor in cultured astrocytes (Kawagishi, Mori, Uno, Kimura, & Chiba, 1994) and to improve mild cognitive impairment in humans (Mori, Inatomi, Ouchi, Azumi, & Tuchida, 2009). Interestingly, Hiwatashi et al. (2010) reported that an ethanol extract of Yamabushitake mushroom had high agonistic activity of PPARα and significantly decreased body weight gain, fat weight, and serum and hepatic triacylglycerol levels with up-regulation of expression of genes related to lipid metabolism in liver of mice. In fact, new ergostane-type sterol fatty acid esters isolated from a methanol extract of the dried fruiting bodies of Yamabushitake showed agonistic activities of all three isotypes of PPARs (PPARα, PPARδ and PPARγ) (Agudelo et al., 2014). Thus, we speculated that intake of Yamabushitake could induce activation of PPARδ in skeletal muscle.

In this study, we examined whether Yamabushitake mushroom had PPARδ agonistic activity in vitro (experiment 1). Then, we measured the expression of PPARδ target genes in skeletal muscle of mice-fed Yamabushitake mushroom (experiment 2). Finally, we examined the effects of intake of Yamabushitake mushroom for 8 weeks on muscle functions and fiber-type composition in mice (experiment 3).

2 | MATERIALS AND METHODS

2.1 | Animals

All animal experiments were conducted in strict accordance with the recommendations in the *Guidelines for Proper Conduct of Animal Experiments* published by the Science Council of Japan, and with the approval of the Animal Care and Use Committee of Kyushu University (approval numbers: A22-148 and A24-138). Male C57BL/6J mice (8 week old) were purchased from a commercial supplier (KBT Oriental Co. Ltd., Tosu, Japan). Mice were housed in plastic cages in an animal room at 22 ± 2°C and 50% ± 10% humidity under an artificial lighting system of 12-hr light and 12-hr darkness (lights on from 08.00 to 20.00 hr). Mice received water and a modified AIN-93G diet (Reeves, 1997) ad libitum for 1 week to acclimate to the environment.

2.2 | Experiment 1: Measurement of PPARδ agonistic activity

2.2.1 | Preparation of Yamabushitake mushroom extracts

We prepared three types of Yamabushitake mushroom extract as described below for the PPARδ ligand activity assay: an ethanol extract (Yama-E), a water extract (Yama-W), and a hexane extract (Yama-H). Freeze-dried ground fruit body of Yamabushitake mushroom (protein 15.5%, lipid 3.7%, carbohydrate 68.9%, ash 6.8%, moisture 5.1%, dietary fiber 45.4%) was immersed in ethanol (10 ml of ethanol per 1 g of dry powder) with shaking for 1 days; then, the suspension was filtered by vacuum filtration. This process was repeated twice more. The collected filtered ethanol was dried using a vacuum desiccator to evaporate ethanol, and the final product was designated Yama-E. Yama-E was immersed in *n*-hexane with shaking for 1 day and the suspension was filtered by vacuum filtration; this process was repeated twice. The collected filtered *n*-hexane was dried in the same manner as Yama-E to yield Yama-H. The residue after treatment in ethanol was suspended in ultrapure water with shaking for 4 hr and the supernatant was filtered after centrifuge separation (800 × *g*, 10 min). This process was repeated once more. The collected filtered ultrapure water was freeze-dried, and the final product was Yama-W.

2.2.2 | PPARδ ligand assay

The agonistic activity of PPARδ was measured by using a nuclear receptor cofactor assay system (EnBio RCAS for PPARδ; Fujikura Kasei Co. Ltd., Tokyo, Japan) following the manufacturer's instruction. Briefly, a peptide of cyclic AMP response element-binding protein (CBP) was immobilized on the bottom of a microplate. After adding the recombinant human PPARδ solution to the wells, dimethyl sulfoxide (DMSO) as the vehicle or extract (Yama-E, Yama-H, or Yama-W) dissolved in DMSO was added, which was incubated for 1 hr at room temperature. The ratio of PPARδ solution to DMSO was 95:5. The final concentration of these extracts was 0.05% (w/v). The binding of PPARδ–ligand complex to the CBP on the plate was detected by horseradish peroxidase (HRP)-conjugated antibody (anti-PPARδ-HRP). The HRP activity was measured by the addition of 3,3′,5,5′-tetramethylbenzidine substrate solution. The absorbance at 450 nm was measured with a microplate reader. As a positive control, 6.25 μmol/L GW501516 (Enzo Life Sciences, Farmingdale, NY, USA), a selective PPARδ agonist, was used. The intensity of PPARδ agonistic activity was calculated as the relative value of the GW501516 set as 100% (B/B max).

2.3 | Experiment 2: Short-term effects of dietary Yamabushitake mushroom intake

2.3.1 | Experimental design

Following the acclimation period, male C57BL/6J mice were divided into two groups (n = 4/group) such that the initial body weight of the two groups was similar. Each diet was prepared based on a modified AIN-93G composition with supplementation of 5% (w/w) Yamabushitake mushroom dry powder (5% Yama) or standard AIN-93G diet (control), the compositions of which are shown in Table 1. Fresh fruit bodies of Yamabushitake mushroom were supplied by Aso Bio-Tech Co. Ltd. (Minamiaso, Japan) and freeze-dried to make a dry powder. Mice received water and one of the two diets ad libitum for 24 hr. Then, EDL muscles were obtained immediately after the mice were killed by decapitation following sevoflurane inhalation anesthesia.

2.3.2 | RNA isolation and real-time quantitative RT-PCR

Total RNA was extracted from whole one-sided EDL muscles of mice using Trizol reagent (Life Technologies, Carlsbad, CA, USA) and reverse transcribed using SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) and Oligo d(T)16 primer (Applied Biosystems, Waltham, MA, USA) according to the manufacturer's protocol. Real-time quantitative PCR, using a LightCycler 1.5 (Roche Diagnostics, Rotkreuz, Switzerland) was performed following the intercalator-based method using specific primers (see Table 2) and EvaGreen dye (Biotium Inc., Fremont, CA, USA). Amplicon specificity was verified by melting curve analysis and a preliminarily tested Taqman probe assay. The threshold cycle (Ct) was defined as the PCR cycle at which an increase in fluorescence above a baseline signal was first detected. The annealing temperature was set to 60°C in all cases. Genes were analyzed using a standard curve constructed from a serial dilution of cDNA aliquots pooled from one randomly chosen sample. The values were standardized to the expression level of hypoxanthine guanine phosphoribosyl transferase (HPRT).

TABLE 1 The composition of experimental diets (g/kg)

Ingredient	Control diet	5% Yamabushitake mushroom diet
Yamabushitake mushroom dry powder	–	50
Corn starch	397.49	347.49
Milk casein	200	200
α-Corn starch	132	132
Sucrose	100	100
Corn oil	70	70
Cellulose powder	50	50
Mineral mix (AIN-93G-MX)	35	35
Vitamin mix (AIN-93-VX)	10	10
L-Cystine	3.0	3.0
Choline bitartrate	2.5	2.5
tert-Butylhydroquinone	0.014	0.014

TABLE 2 Primers used for real-time quantitative PCR

mRNA		Sequence (5′-3′)	Amplicon (nt)	Access no.
Pdk4	Forward	CGCTTAGTGAACACTCCTTCG	94	NM_013743.2
	Reverse	CTTCTGGGCTCTTCTCATGG		
Ucp3	Forward	TACCCAACCTTGGCTAGACG	77	NM_009464.3
	Reverse	GTCCGAGGAGAGAGCTTGC		
Hprt1	Forward	TCCTCCTCAGACCGCTTTT	90	NM_013556.2
	Reverse	CCTGGTTCATCATCGCTAATC		

Pdk4, pyruvate dehydrogenase kinase, isoenzyme 4; *Ucp3*, uncoupling protein 3; *Hprt1*, hypoxanthine guanine phosphoribosyl transferase 1.

2.4 | Experiment 3. Long-term effects of dietary Yamabushitake mushroom intake

2.4.1 | Experimental design

Male C57BL/6J mice were divided into two groups (n = 7 or 8/group) in the same manner as for experiment 2. Mice were given each diet for 8 weeks by pair-feeding and allowed free access to water during the entire experimental period. Food intake and body weight were measured daily. The mice were killed after the 8-weeks feeding period following the same procedure as in experiment 2.

2.4.2 | Tetanic force measurements

In week 8 of the feeding period, maximum isometric plantar-flexion force torque was measured with a perpendicularly fixed ankle under continuous sevoflurane anesthesia by an anesthetic vaporizer Narcobit-E(II) (Natsume Seisakusho Co. Ltd., Tokyo, Japan), according to the method described in Iwata, Fuchioka, Hiraoka, Masuhara, and Kami (2010) with some modifications (Mizunoya et al., 2015). Briefly, a set of four strain gauges attached to a Bakelite footplate base was connected to an instrumentation amplifier and a MacLab/4S digital converter (ADInstruments, Dunedin, New Zealand), which is able to detect force exerting to the footplate. The force was calibrated by a standard curve of several weights. Tetanic contractions by the posterior muscle in the murine lower hind limb (right side) were generated by an electrical tibial nerve stimulation via a bipolar hook-shaped electrode (KS212-012; Unique Medical Co. Ltd., Tokyo, Japan). The optimal conditions of stimulation to induce tetanus (amplitude 60 V, rectangular pulse width 1 ms with intervals of 3 ms after each pulse, frequency 250 Hz, duration 160 ms) was determined in advance in order to generate maximum isometric force. Muscle endurance was evaluated by successive electrical stimulations every 1 s, and force was recorded for 100 s (100 times stimulus). Force measurements were converted to torque by multiplying the distance between the medial malleolus and the head of the first metatarsal bone (Mt_1) for each mouse.

2.4.3 | Analysis of MyHC isoform composition

Protein samples extracted from quadriceps muscle were subjected to high-resolution sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to assess MyHC isoform composition, as described in detail by Mizunoya, Wakamatsu, Tatsumi, and Ikeuchi (2008). Briefly, protein samples (100 ng) were loaded on a gel composed of 8% acrylamide (acrylamide/bis ratio, 99:1) with 35% (v/v) glycerol. Then, electrophoresis was performed at a constant voltage of 140 V for 22 hr at 4°C. Gels were stained with Silver Stain Kanto III (Kanto Chemical Co. Inc., Tokyo, Japan) and dried. The bands were captured on an image scanner, and the relative content of MyHC isoforms was quantified by densitometry, using ImageJ 1.34s software (National Institutes of Health, Bethesda, MD, USA).

2.5 | Statistical analysis

Student's t-test was used for statistical analysis of experimental results using Excel X for Macintosh (Microsoft Corp., Redmond, WA, USA). Data are represented as $M \pm SE$). The level of significance was set to $p < 0.05$ throughout the study, and statistically significant differences from the vehicle (Figure 1) and control group (Figures 2 and 3, and Table 3) at $p < 0.05$ and $p < 0.01$ are indicated by * and **, respectively.

3 | RESULTS

3.1 | Experiment 1: PPARδ agonistic activity of extracts from Yamabushitake mushroom

First, PPARδ agonistic activities of various Yamabushitake mushroom extracts (Yama-W, Yama-E, and Yama-H) were measured. The 0.05% (w/v) Yama-E and Yama-H extracts showed about 20% (B/B max) agonistic activity, and the activity of Yama-H extract was statistically different compared with vehicle (Figure 1). The agonistic activity of Yama-W extract was also significantly higher than that of vehicle, but was only about 10% (B/B max).

3.2 | Experiment 2: mRNA expression of PPARδ target genes in skeletal muscle

To examine whether skeletal muscle PPARδ activation was induced in mice by dietary Yamabushitake mushroom intake, we analyzed mRNA expression of two PPARδ target genes, pyruvate

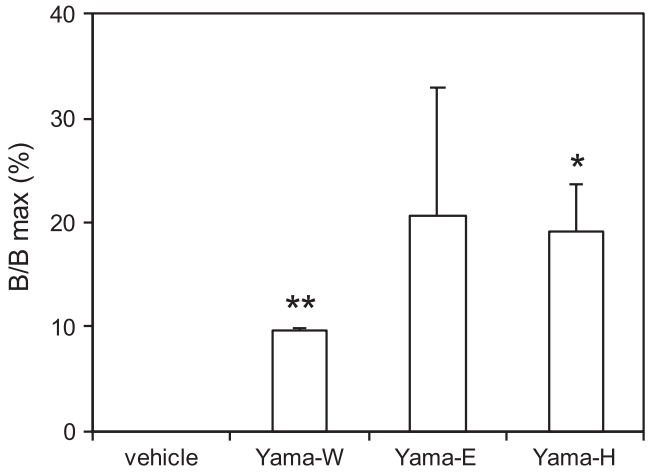

FIGURE 1 Peroxisome proliferator-activated receptor (PPAR) δ agonistic activity of Yamabushitake mushroom extracts as measured by a nuclear receptor cofactor assay system (EnBio RCAS for PPARδ by Fujikura Kasei Co., Ltd., Tokyo, Japan). GW501516, a selective PPARδ agonist, was used as positive control. Relative activity (B/B max) is shown, with the maximum activity (B max) of GW501516 being 100%. Values of vehicle and 0.05% (w/v) extracts of Yamabushitake mushroom are means of triplicate analysis. Yama-W = water extract, Yama-E = ethanol extract, Yama-H = hexane extract. Statistical differences were evaluated using *t*-tests compared with vehicle values. Asterisks indicate significant differences compared with vehicle: $^{*}P < 0.05$, $^{**}P < 0.01$

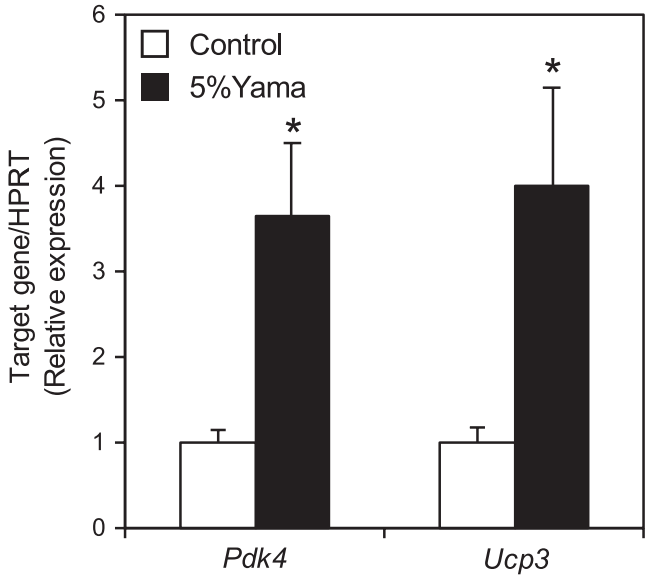

FIGURE 2 Relative mRNA expression of peroxisome proliferator-activated receptor (PPAR)δ target genes *Pdk4* and *Ucp3* in extensor digitorum longus muscles of control mice and mice fed 5% Yamabushitake mushroom for 24 hr. Gene expression was normalized to mRNA expression level of hypoxanthine guanine phosphoribosyl transferase (HPRT). Values ($M \pm SE$; $n = 4$). are expressed as fold changes compared with control mice. Statistical differences were evaluated using *t*-tests. Asterisks (*) indicate significant differences compared with control at $P < 0.05$

dehydrogenase kinase 4 (*Pdk4*) and uncoupling protein 3 (*Ucp3*), by real-time quantitative RT-PCR in murine skeletal muscle after short-term (24-hr) intake of Yamabushitake mushroom. The mRNA expression of *Pdk4* and *Ucp3* was significantly up-regulated in EDL muscle of mice in the 5% Yama group compared with that of control group mice (Figure 2).

3.3 | Experiment 3: Growth profiles of animals

Results of experiments 1 and 2 showed that Yamabushitake mushroom contained agonistic substrates for PPARδ. To examine the effect of longer-term intake of Yamabushitake mushroom on muscle function and fiber type, mice were fed a diet supplemented with 5% Yamabushitake for 8 week. The growth profiles for body weight, weights of lower hind-limb muscles (soleus, plantaris, gastrocnemius, tibialis anterior, and EDL), and weights of other tissues in the control and 5% Yama groups are shown in Table 3. None of the parameters except heart weight differed significantly between the two groups. Heart weight was significantly lower in mice in the 5% Yama group than in the control group.

3.4 | Experiment 3: Improvement of muscle endurance

The initial tetanic torques at the first stimulation (0 s), assumed to be maximum muscular force, were equivalent between the control and 5% Yama groups. The resistance to fatigue (declining torque over time) was significantly greater in the 5% Yama group than in the control group (Figure 3a; at 16, 38, 41–44, 47, and 49 s). Area under the curve (AUC) of torque during the 100-s stimulation, which is thought to represent the total ability of muscle endurance, tended to be higher in the 5% Yama group ($p = 0.0625$; Figure 3b).

3.5 | Experiment 3: Analysis of MyHC isoform composition

To determine whether the observed increase in muscle endurance resulted from alterations in muscle fiber-type composition following dietary Yamabushitake mushroom intake, we performed an optimized SDS-PAGE to examine the composition of MyHC isoforms. In quadriceps muscles, silver-stained protein bands indicating MyHC 2A + 2X (intermediate fibers) and 2B (fast-twitch fibers) were detected. As shown in Figure 4, we observed no significant difference in composition of MyHC 2A + 2X and 2B isoforms between the 5% Yama and control groups.

4 | DISCUSSION

To date, scientific reports revealing PPARδ agonistic activity from natural food components have been scarce. In this study, we found that extracts from Yamabushitake mushroom had agonistic activity on PPARδ. The ethanol and hexane extracts had higher activity

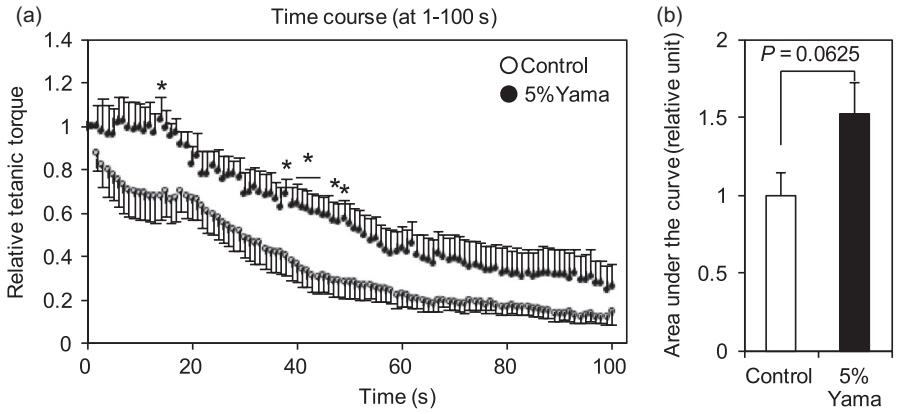

FIGURE 3 Muscle endurance abilities of anesthetized mice after being fed a diet supplemented with 5% Yamabushitake mushroom for 8 wks. Muscle endurance was measured by successive isometric plantar-flexion force torque, which was generated by electrical stimulation (amplitude 60 V, rectangular pulse width 1 ms with intervals of 3 ms after each pulse, frequency 250 Hz, duration 160 ms) to the right tibial nerve branch that innervates the lower hind-limb muscles. Electrical stimulations were induced every second and the produced force was recorded for 100 s (100 times stimulus). The initial tetanic contraction force was set to a value of 1 in each group. Values are the *M* ± *SE* for 7–8 mice. Statistical differences were evaluated using *t*-tests. Asterisks (*) indicate significant difference compared with control at $p < 0.05$

than the water extract in vitro (Figure 1), suggesting that the agonistic substrates in Yamabushitake mushroom are lipophilic. These data are in agreement with previous reports that PPARδ is activated by natural lipophilic ligands such as fatty acids and their derivatives (Forman, Chen, & Evans, 1997; Krey, 1997). The ethanol extract of Yamabushitake mushroom might contain PPARδ agonistic substances; however, its activity was not significantly higher compared with vehicle and thus we expected that the PPARδ agonistic substances were concentrated by hexane extraction. To date, new ergostane-type sterol fatty acid esters have been the leading candidates for PPARδ agonistic substances contained in Yamabushitake mushroom (Agudelo et al., 2014).

Mice fed a diet supplemented with 5% Yamabushitake mushroom for 24 hr showed significant up-regulation of mRNA expression of *Pdk4* and *Ucp3* in skeletal muscle; *Pdk4* and *Ucp3* are known PPARδ target genes (Degenhardt et al., 2007; Villarroya, Iglesias, & Giralt, 2007). This result suggested that oral intake of Yamabushitake mushroom activates PPARδ of skeletal muscle in vivo. The PDK4 protein is thought to play a key role in regulating fat and glucose substrate utilization in metabolic tissues. It also facilitates fatty acid oxidation by inactivating the pyruvate dehydrogenase complex to switch energy production from the glycolytic pathway to β-oxidation (Manickam & Wahli, 2017; Zhang, Hulver, McMillan, Cline, & Gilbert, 2014). The UCP3 protein is mainly expressed in skeletal muscle; however, it is also found in brown adipose and heart tissue and plays important roles in mitochondrial fatty acid oxidation and thermogenesis (Busiello, Savarese, & Lombardi, 2015). PDK4 and UCP3 are also known as mitochondrial metabolic protein in skeletal muscle. The result of increase of *Pdk4* and *Ucp3* expression suggested that oral intake of Yamabushitake mushroom improved mitochondrial function and it caused enhancement of muscle endurance in 8-wk supplementation of Yamabushitake mushroom.

TABLE 3 Growth performance and organ weights in mice fed each diet

	Control	5% Yama
Initial body weight (g)	24.4 ± 0.4	24.1 ± 0.2
Final body weight (g)	24.5 ± 0.6	23.9 ± 0.5
Soleus (mg)	7.71 ± 0.29	7.57 ± 0.56
Plantaris (mg)	16.7 ± 1.1	14.7 ± 1.27
Gastrocnemius (mg)	127.0 ± 5.8	119.2 ± 6.1
TA (mg)	34.0 ± 3.9	39.9 ± 2.3
EDL (mg)	10.1 ± 0.70	9.43 ± 0.89
Epididymal fat	233.3 ± 45.0	274.5 ± 32.1
Perirenal fat	68.7 ± 8.1	68.6 ± 9.7
Liver	822.9 ± 32.7	823.8 ± 12.4
Kidneys	290.3 ± 16.7	263.5 ± 5.4
Spleen	57.9 ± 1.8	55.5 ± 2.7
Heart	118.7 ± 4.7	107.5 ± 2.0[a]

Values are *M* ± *SE* for 7–8 mice.
TA; tibialis anterior, EDL; extensor digitorum longus.
[a]Significantly different from control group ($p < 0.05$).

PPARδ is a modulator of muscle fiber-type composition, which induces the transition from fast to slow fiber type (Wang et al., 2004). In our experiments, we observed no significant difference in the composition of MyHC isoforms, which are typical fiber-type marker proteins, between control and Yamabushitake mushroom-fed mice, despite the up-regulated expression of PPARδ target genes. Previous studies that showed a drastic increase in slow-twitch fibers used constitutively activated PPARδ overexpression mice (Gan et al., 2013; Wang et al., 2004). We initially thought that PPARδ overexpression and PPARδ activation of agonist had similar outcomes; therefore, we assumed that dietary intake of Yamabushitake mushroom could induce the fast-to-slow muscle fiber-type transition. In

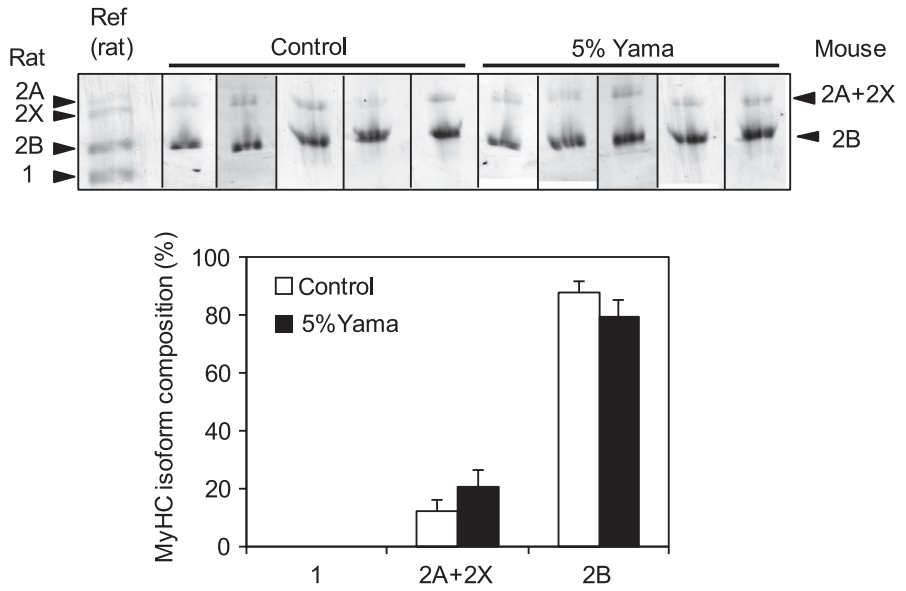

FIGURE 4 Composition of myosin heavy chain (MyHC) isoforms in quadriceps muscles in mice fed a diet supplemented with 5% Yamabushitake mushroom (5% Yama) for 8 weeks. Isoform composition was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using quadriceps muscles from control and 5% Yama mice. The bands represent the MyHC isoforms (2A + 2X and 2B) detected in high-resolution silver-stained SDS-PAGE gels for five representative mice from each group. The lanes were sorted into experimental groups from the original random assignment. A mix of rat extensor digitorum longus muscles and soleus samples were used as the four MyHC isoform references (2A, 2X, 2B, and 1; Ref; left lane). The graph shows the composition of MyHC isoforms in control and 5% Yama mice. Values are *M* ± *SE* for 7 to 8 mice

fact, this assumption could be incorrect. Previously, Narkar et al. (2008) reported that activation of PPARδ by its specific agonist alone did not increase slow-twitch fibers in mice though oxidative metabolism was enhanced, meanwhile a combination of PPARδ agonist treatment and exercise training resulted in an obvious increase in type 1 fibers. Thus, activation of PPARδ by supplementation of Yamabushitake mushroom might not be sufficient to lead to an apparent change in the skeletal muscle fiber type.

As another possibility, we thought that PPARα could be activated, in addition to PPARδ, because PPARα is expressed in skeletal muscle, and *Pdk4* and *Ucp3* are also target genes of PPARα (Escher et al., 2001; Mandard, Muller, & Kersten, 2004). Hiwatashi et al. (2010) reported that an ethanol extract from Yamabushitake mushroom had higher agonistic activity of PPARα, indicating that Yamabushitake mushroom could contain both PPARα and PPARδ ligand substrates. Interestingly, overexpression of PPARα increases fast-twitch fibers, not slow-twitch fibers (Gan et al., 2013). Therefore, simultaneous activation of PPARδ and PPARα might result in a competitive effect on muscle fiber-type composition, resulting in no overall alteration of muscle fiber-type composition.

The 8-weeks supplementation with 5% Yamabushitake mushroom significantly increased muscle endurance capacity in mice (Figure 3a). Several studies have shown that dietary components improve the systemic endurance capacity of mice. Murase et al. reported that a combination of swimming exercise and dietary green tea extract significantly prolonged swimming time in mice compared with swimming exercise alone (Murase, Haramizu, Shimotoyodome, Nagasawa, & Tokimitsu, 2005). In another study, Kim et al. reported that administration of capsaicin increased swimming time in mice via secretion of adrenal catecholamine (Kim, Kawada, Ishihara, Inoue, & Fushiki, 1997). Although these studies examined systemic endurance rather than muscle endurance capacity, we expected that an increase in muscle endurance caused by these dietary components might explain the increased systemic endurance. In our study, dietary Yamabushitake mushroom intake significantly improved muscle endurance (Figure 4). A recent study showed that activation of PPARδ promoted running endurance by preserving glucose utilization during exercise (Fan et al., 2017). In that report, the authors noted that *Pdk4* was a mitochondrial gatekeeper gene because it negatively regulated the influx of glucose-derived pyruvate into the mitochondrial tricarboxylic acid cycle. That function probably spared muscle glycogen consumption during exercise, leading to improved endurance capacity. In our study, the expression of *Pdk4* was up-regulated by supplementation with Yamabushitake mushroom for 24 hr. Thus, the up-regulation of *Pdk4* expression via PPARδ activation contributed to the increase in muscle endurance in mice fed Yamabushitake mushroom for 8 weeks, possibly regardless of muscle fiber-type composition.

5 | CONCLUSIONS

We found that extracts from Yamabushitake mushroom had PPARδ agonistic activity in vitro, and that supplementation of mice with 5% Yamabushitake mushroom for 24 hr up-regulated the expression of the PPARδ target genes *Pdk4* and *Ucp3* in vivo. Furthermore,

8-weeks supplementation with 5% Yamabushitake mushroom increased muscle endurance in lower hind-limb muscles of mice, possibly via PPARδ activation. Although muscle fiber-type composition was not altered by intake of Yamabushitake mushroom, activation of PPARδ and the increase in muscle endurance may contribute to the technology of controlling animal metabolism and physical activity in the future.

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