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A. ZEMBRON-LACNY[1], M. GAJEWSKI[2], M. NACZK[3], I. SIATKOWSKI[4]

EFFECT OF SHIITAKE (*LENTINUS EDODES*) EXTRACT ON ANTIOXIDANT AND INFLAMMATORY RESPONSE TO PROLONGED ECCENTRIC EXERCISE

[1]Department of Sports Medicine, Faculty of Physical Culture in Gorzow Wielkopolski, University School of Physical Education, Poznan, Poland; [2]Department of Infectious Diseases, Medical University of Gdansk, Gdansk, Poland; [3]Department of Physiology, Faculty of Physical Culture in Gorzow Wielkopolski, University School of Physical Education, Poznan, Poland; [4]Department of Mathematical and Statistical Methods, University of Life Sciences Poznan, Poland

The shiitake (*Lentinus edodes*) extract is purported to have potent antioxidant, anti-inflammatory and regenerative properties due to presence of many bioactive compounds such as ergothioneine. This study was designed to assess the antioxidant and anti-inflammatory activity of shiitake extract in healthy men exposed to exercise-induced skeletal muscles damage. Subjects ingested shiitake mushroom extract (700 mg, two times per day) or placebo for 10 days prior to two separate exercise trials (crossover study). The exercise session involved 90 min run at 65% VO_2max (0% gradient) and 15-min eccentric phase at 65% VO_2max (-10% gradient). Subjects experienced creatine kinase (peak 461±206 IU/L) and leukocytes (peak 9.82 × 10^3/μL) elevations indicating muscle damage and inflammation. Exercise altered plasma IL-6 (peak 5.29±0.78 pg/mL), IL-10 (peak 24.75±6.22 pg/mL) and IL-1β (peak 0.54±0.09 pg/mL) levels but did not affect tumour necrosis factor α (TNF-α) level relative to baseline. Shiitake extract did not demonstrate any effect on immune cells number and inflammatory mediators level, with the exception of IL-10. Thiol redox status (GSH_{total}–2GSSG/GSSG) and niric oxide (NO) concentration increased after shiitake extract whereas H_2O_2 and 8-isoprostanes did not change. In conclusion, shiitake mushroom extract had no effect on markers of inflammation following prolonged eccentric exercise but demonstrated an antioxidant activity through the regulation of nitric oxide concentration and thiol redox status.

Key words: *excercise, ergothioneine, cytokines, glutathione, hydrogen peroxide, nitric oxide, shiitake, superoxide dismutase*

INTRODUCTION

Shiitake (*Lentinus edodes*) is one of the most popular edible mushrooms in the world, production globally being second only to the button mushrooms *Agaricus bisporus*. Interest is increasing in its high nutritional values and medicinal properties, traditionally acknowledged by oriental cultures. Compounds produced by shiitake which are attributed to have antioxidant, antimicrobial, antilipidemic, anticancer, anticariogenic and immunoregulatory activities include lentinan, eritadenine, ergothioneine as well as vitamins, especially provitamin D_2 (ergosterol and carciferol), vitamins B (thiamine, riboflavin and niacin) and panthothenic acid. Minerals found at high concentrations include magnesium, nickel, copper, phosphorus, strontium and zinc (1-5). Shiitake mushrooms are becoming popular in nutritional (called functional food) and medical products through Asia, Europe, and North America (4). However, shiitake has not as yet been assessed for the health benefits of its oral administration in physically active men.

The antioxidant effect of shiitake is caused by L-ergothioneine (2-mercaptohistidine trimethylbetaine) ranging 1.98±0.11 mg/g dry-weight (6). Weigand-Heller *et al*. (5) demonstrated the significant increase in blood ergothioneine during two hours after the mushroom consumption. Ergothioneine is present from 100 μM to 2 mM in human tissues predisposed to high levels of oxidative stress and inflammation such as liver, kidney, erythrocytes, seminal fluid, eye lens and skeletal muscles (7-9). The antioxidant and anti-inflammatory properties of ergothioneine are related to its ability to trap reactive oxygen/nitrogen species and inflammatory mediators such as hydroxyl radicals, hypochlorous acid and peroxynitrite ($ONOO^-$) which is formed endogenously by reaction of nitric oxide (NO) with superoxide (O_2^-). Another antioxidative attribute of ergothioneine is the intermediate value of the standard redox potential of the thiol/disulfide couple (–0.06V) compared with other naturally occurring thiols (from –0.2 to –0.32V). This property confers greater stability under physiological conditions, hence ergothioneine does not readily undergo auto-oxidation as rapidly as other antioxidant thiols such as glutathione or lipoic acid which can generate hydrogen peroxide (H_2O_2) in the process (1). Numerous *in vitro* studies have demonstrated the antioxidant and anti-inflammatory capabilities of various antioxidants such as vitamin C, vitamin E, lipoic acid and

flavonoids, but an antioxidant role of ergothioneine has yet to be fully verified *in vivo*.

Currently, reactive oxygen/nitrogen species H_2O_2 and NO are perceived as important signalling molecules generated during muscle contraction and involved in the regeneration and adaptation of skeletal muscle to physical work (10, 11). H_2O_2 and NO are produced during muscle work by the enzymes superoxide dismutase (isoforms CuZnSOD and MnSOD) and nitric oxide synthase (isoforms nNOS, eNOS and iNOS) which are localized to the muscle sarcolemma and mitochondria (12, 13). The studies in human isolated muscle and myotube culture demonstrated that H_2O_2 and NO produced within contracting skeletal muscle are key regulators of pre- and posttranslational signalling events leading to cytokines expression (11, 14).

The production of cytokines is not directly induced by H_2O_2 and NO but by thiol compounds, mainly glutathione. The ratio of reduced (GSH) to oxidized glutathione (GSSG) plays an essential role in regulating thiol-dependant signalling pathways. Exercise-induced changes in thiol redox status lead to conformational changes in transcriptional factors, release of inhibitory subunits, or promotion of protein complex formations which are necessary for signal transduction or transcription to proceed (15). Ergothioneine is able to regulate the cytokine synthesis by a mechanism depending on its thiol-mediated antioxidant property (16).

Shiitake extract, as a primary source of ergothioneine, could have the potential to modulate H_2O_2, NO and cytokine levels and to enhance the skeletal muscle regeneration after intense exercise. Therefore, we designed our study to evaluate the antioxidant and anti-inflammatory effect of shiitake extract in relation to thiol redox status in healthy men exposed to exercise-induced muscle damage.

MATERIAL AND METHODS

Fourteen healthy males (age 21.0±0.9 years, height 1.8±0.1 m, body mass 73.0±6.5 kg, VO_2max 58.0±4.7 mL/kg/min) participated in the placebo-controlled and crossover study. During the study, subjects were requested not to use nutritional supplements as well as to avoid physical effort for 48 hours before and after the exercise session. All the subjects were informed of the aim of the study and gave their written consent for participation in the project. The protocol of the study was approved by the local ethics committee in accordance with the Helsinki Declaration.

The subjects were assigned to one of two groups: shiitake or placebo group, and performed two separate exercise sessions. The wash-out period between the first and the second phase of the study was three weeks. Subjects ingested shiitake mushroom (*Lentinus edodes*) extract (700 mg, two times per day, Laboratorios Bio-Dis Espana, Sevilla, Spain) or placebo for 10 days prior to exercise. The exercise session involved 90-min run at 65% VO_2max (0% gradient) and 15-min eccentric phase at 65% VO_2max (–10% gradient) on a motor-driven treadmill. Blood samples were obtained from antecubital vein to single-use containers with an anticoagulant ($EDTAK_2$) at pre-exercise and post-exercise periods (20 min, 24 and 48 hours). After collection, the samples were immediately placed at 4°C temperature. Within 10 min, they were centrifuged at 3000 g and 4°C for 10 min. Aliquots of plasma were stored at –80°C.

Muscle damage

Plasma creatine kinase (CK) activity was used as a marker of muscle damage and was evaluated by Emapol kit (Poland). CK detection limit for the applied kit was 6 U l^{-1}. The intra-assay CV for the CK kit was 1.85%.

Reactive oxygen/nitrogen species (RONS)

Plasma hydroperoxide (H_2O_2) and nitric oxide (NO) concentrations were determined using Oxis Research kit (USA). H_2O_2 and NO levels were measured immediately after plasma collection, on the day of exercise study. H_2O_2 and NO detection limits were 6.25 µM and 0.5 µM respectively. The intra-assay coefficient of variation (CV) for the H_2O_2 kit and for the NO kit it was <10%. Plasma 8-isoprostane level, as a marker of RONS activity, was measured with Cayman kit (USA). 8-Isoprostane detection limit for the procedure was 2.7 pg/mL, and the intra-assay coefficient of variation (CV) was 6.4%.

Thiol redox status

Total (GSH_t) and oxidized glutathione (GSSG) were measured with Oxis Research kit (USA). The concentrations of GSH_t and GSSG were calculated using reduced glutathione as a standard and the results were expressed in µmol/L. Detection limits for the GSH and GSSG were 0.1 µmol/L and 0.02 µmol/L, respectively. The intra-assay CV for GSH and GSSG were 0.96% and 6.45%, respectively. Thiol redox status was calculated according to the following equation: (GSH_t-2GSSG)/GSSG. Before the measurement of glutathione, the blood samples were protected from oxidation according to the protocol of Oxis Research.

Pro- and anti-inflammatory cytokines

Plasma interleukin-1β (IL-1β), tumour necrosis factor α (TNF-α), interleukin-6 (IL-6) and interleukin-10 levels were determined by enzyme immunoassay methods using commercial kits (R&D Systems, USA). Detection limits for IL-1β TNF-α IL-6 and IL-10 were 0.023, 0.038, 0.039 and 0.500 pg/mL, respectively. The average intra-assay CV was about 8.0% for all cytokines.

Plasma volume

Haemoglobin (Hb) and haematocrit (Hct) levels as well as immune cells number were assessed using Sysmex K-4500 (Kobe, Japan). The post-exercise values were corrected for changes in plasma volume according to Kraemer and Brown (17). Relative changes in plasma volume were calculated according to the following equation:

$$\%\Delta PV = 100 \times \{([Hb]_1/[Hb]_2) \times [100 - (Hct_2 \times 0.874)] / [100 - (Hct_1 \times 0.874)] - 1\},$$

where $[Hb]_1$ (g dl^{-1}) and Hct_1 (%) are mean initial values, $[Hb]_2$ and Hct_2 are post-exercise values. The Hct was multiplied by 0.96 and 0.91 to correct for trapped plasma and peripheral sampling respectively (18).

Statistical analysis

A two-way analysis of variance (ANOVA) with repeated measures was used to determine the effect of exercise and shiitake extract on analysed parameters [(pre- *vs*. post-exercise) × (placebo *vs*. shiitake)]. Tukey's post-hoc test was used to determine differences between group means. The relationships between variables were determined using Pearson's correlation and linear regression. All results are expressed as mean and standard deviation (× ±S.D.). Statistical significance was set at $P<0.05$. Statistical analysis was performed using Statistica 9.0 and the statistical package R2.10.0 (R Development Core Team 2009).

RESULTS

Muscle damage and immune cells (Table 1)

Total CK activity and immune cells number increased after exercise indicating muscle damage and inflammation. Shiitake extract increased in rest CK activity however, differences between shiitake and placebo were not detected at post-exercise period. No effect of shiitake on CK activity suggests that shiitake extract does not improve the skeletal muscle regeneration during recovery. Immune cells number did not demonstrate any differences between shiitake and placebo. The number of immune cells directly correlated with levels of H_2O_2 and NO which are mediators of inflammatory reaction (*Table 2*).

Reactive oxygen/nitrogen species (Table 3)

Shiitake extract elevated H_2O_2 and NO concentrations at 20 min after exercise session. However, H_2O_2 did not demonstrate any differences between shiitake and placebo whereas NO was significantly higher after shiitake extract ingestion before and 20 min after exercise. Shiitake reduced 8-iso concentration at 20 min and 24 hours after exercise compared with placebo but the differences were not significant.

Thiol redox status (Table 3)

Shiitake extract did not affect GSH_t but it reduced GSSG concentration which tended towards to low values during recovery. The changes in GSSG affected thiol redox status at pre-exercise (*Fig. 1*).

Pro- and anti-inflammatory cytokines (Table 4).

Two typical pro-inflammatory cytokines IL-1β and TNF-α increased at 24 h after exercise, similarly to CK activity. Shiitake extract reduced IL-1β and TNF-α at 24 h post-exercise, however, differences were not significant. IL-6 and IL-10 simultaneously increased at 20 min after exercise. IL-6 did not change after shitake extract whereas IL-10 was significantly higher in shiitake group compared with placebo. IL-6 and IL-10 highly correlated with neutrophils number (*Table 2*), and then IL-6 and IL-10 correlated with NO (r=0.520, $P<0.01$; r=0.616, $P<0.001$). This means that immune cells are an important source

Table 1. Effect of exercise and shiitake extract on activity of creatine kinase (CK) and number of immunological cells.

	Pre-exercise	**Shiitake *vs*. Placebo**	**Post-exercise 20 min**	**Shiitake *vs*. Placebo**	**Post-exercise 24 h**	**Shiitake *vs*. Placebo**	**Post-exercise 48 h**	**Shiitake *vs*. Placebo**
CK IU/L Shiitake extract Placebo	229 ± 48 135 ± 35	*P<0.001*	303 ± 49 230 ± 78	ns	527 ± 259* 461 ± 206*	ns	304 ± 107 309 ± 112	ns
Leukocytes × 10^3/µL Shiitake extract Placebo	6.60 ± 1.12 5.48 ± 1.42	ns	11.84 ± 3.12** 9.82 ± 1.20**	ns	6.83 ± 1.53 5.63 ± 1.30	ns	6.49 ± 1.09 5.60 ± 1.21	ns
Monocytes × 10^3/µL Shiitake extract Placebo	0.52 ± 0.13 0.51 ± 0.20	ns	0.79 ± 0.26** 0.80 ± 0.41**	ns	0.45 ± 0.17 0.59 ± 0.22	ns	0.62 ± 0.16 0.47 ± 0.19	ns
Neutrophils x 10^3/µL Shiitake extract Placebo	4.40 ± 0.89 3.24 ± 0.83	ns	9.70 ± 1.71*** 7.24 ± 1.24**	ns	4.30 ± 1.03 2.96 ± 0.78	ns	4.19 ± 0.81 2.69 ± 0.61	ns
Lymphocytes × 10^3/µL Shiitake extract Placebo	2.00 ± 0.35 1.86 ± 0.46	ns	1.78 ± 0.42 1.81 ± 0.46	ns	1.98 ± 0.45 1.66 ± 0.30	ns	1.80 ± 0.48 1.77 ± 0.34	*ns*

**P<0.05*; ***P<0.01* and ****P<0.001* indicate significant differences pre-exercise *vs*. post-exercise.

Table 2. Statistical relationships (correlation coefficients) between number of immune cells and concentrations of hydrogen peroxide (H_2O_2), nitric oxide (NO) and cytokines IL-6 and IL-10.

		H_2O_2 mmol/mL	**NO mmol/mL**	**IL-6 pg/mL**	**IL-10 pg/mL**
Leukocytes × 10^3/µL	Shiitake extract	0.580 *P<0.01*	0.503 *P<0.01*	0.830 *P<0.001*	0.638 *P<0.01*
	Placebo	0.349 *P>0.05*	0.093 *P>0.05*	0.753 *P<0.001*	0.570 *P<0.001*
Monocytes × 10^3/µL	Shiitake extract	0.395 *P<0.5*	0.532 *P<0.01*	0.600 *P<0.001*	0.450 *P<0.001*
	Placebo	–0.167 *P>0.05*	–0.121 *P>0.05*	0.313 *P>0.05*	0.109 *P>0.05*
Neutrophils × 10^3/µL	Shiitake extract	0.457 *P<0.01*	0.484 *P<0.01*	**0.941** ***P<0.001***	**0.749** ***P<0.001***
	Placebo	0.101 *P>0.05*	0.055 *P>0.05*	**0.856** ***P<0.001***	**0.727** ***P<0.001***
Lymphocytes × 10^3/µL	Shiitake extract	0.049 *P>0.05*	–0.068 *P>0.05*	–0.333 *P>0.05*	–0.213 *P>0.05*
	Placebo	0.073 *P>0.05*	–0.097 *P>0.05*	–0.100 *P>0.05*	–0.295 *P>0.05*

Table 3. Effect of exercise and shiitake extract on concentrations of hydrogen peroxide (H_2O_2), nitric oxide (NO), 8-isoprostane (8-iso), total glutathione (GSH_t) and oxidised glutathione (GSSG).

	Pre-exercise	Shiitake *vs.* Placebo	Post-exercise 20 min	Shiitake *vs.* Placebo	Post-exercise 24 h	Shiitake *vs.* Placebo	Post-exercise 48 h	Shiitake *vs.* Placebo
H_2O_2 mmol/ mL Shiitake extract Placebo	3.42 ± 0.86 4.42 ± 0.56	ns	6.54 ± 1.27*** 5.80 ± 2.83	ns	5.70 ± 0.93*** 4.28 ± 1.29	ns	5.58 ± 0.56*** 7.27 ± 1.7	ns
NO mmol/mL Shiitake extract Placebo	35.56 ± 4.24 25.55 ± 3.81	*P<0.001*	42.63 ± 4.62* 26.74 ± 3.14	*P<0.001*	29.53 ± 4.91 27.77 ± 5.93	*ns*	32.68 ± 5.11 29.02 ± 2.19	*ns*
8-iso pg/mL Shiitake extract Placebo	24.43 ± 7.85 26.41 ± 3.78	ns	27.55 ± 6.93 34.87 ± 5.57*	ns	19.08 ± 6.63 22.82 ± 7.65	ns	26.81 ± 7.71 19.14 ± 4.59	ns
GSH_t mmol /mL Shiitake extract Placebo	1196 ± 113 1327 ± 239	ns	1198 ± 101 1134 ± 279	ns	1390 ± 103* 1167 ± 310	ns	1413 ± 184 1318 ± 116	ns
GSSG mmol /mL Shiitake extract Placebo	4.80 ± 1.04 10.87 ± 3.16	*P<0.01*	6.56 ± 1.49* 9.35 ± 4.40	ns	5.44 ± 0.61 8.30 ± 4.70	ns	5.42 ± 1.00 7.80 ± 4.66	ns

*$P<0.05$; **$P<0.01$ and ***$P<0.001$ indicate significant differences pre-exercise *vs*. post-exercise.

Table 4. Effect of exercise and shiitake extract on concentrations of interleukins IL-6, IL-10, IL-1β and TNF-α.

	Pre-exercise	Shiitake *vs.* Placebo	Post-exercise 20 min	Shiitake *vs.* Placebo	Post-exercise 24 h	Shiitake *vs.* Placebo	Post-exercise 48 h	Shiitake *vs.* Placebo
IL-1β pg/mL Shiitake extract Placebo	0.41 ± 0.17 0.34 ± 0.06	ns	0.43 ± 0.12 0.30 ± 0.06	ns	0.43 ± 0.14 0.54 ± 0.09***	ns	0.35 ± 0.14 0.40 ± 0.09	ns
TNF-α pg/mL Shiitake extract Placebo	2.11 ± 0.26 2.39 ± 0.52	ns	2.36 ± 0.42 2.27 ± 0.30	ns	2.11 ± 0.27 2.85 ± 0.27	ns	1.80 ± 0.23 2.01 ± 0.58	ns
IL-6 pg/mL Shiitake extract Placebo	1.32 ± 0.30 0.92 ± 0.20	ns	5.01 ± 0.92*** 5.29 ± 0.78***	ns	1.54 ± 0.59 1.60 ± 0.98	ns	1.66 ± 0.55 1.13 ± 0.37	ns
IL-10 pg/mL Shiitake extract Placebo	18.87 ± 2.40 13.46 ± 1.48	*P<0.001*	32.65 ± 4.44*** 24.75 ± 6.22**	*P<0.05*	17.99 ± 2.57 17.63 ± 1.89	ns	18.48 ± 1.77 17.61 ± 1.26	ns

*$P<0.05$; **$P<0.01$ and ***$P<0.001$ indicate significant differences pre-exercise *vs*. post-exercise.

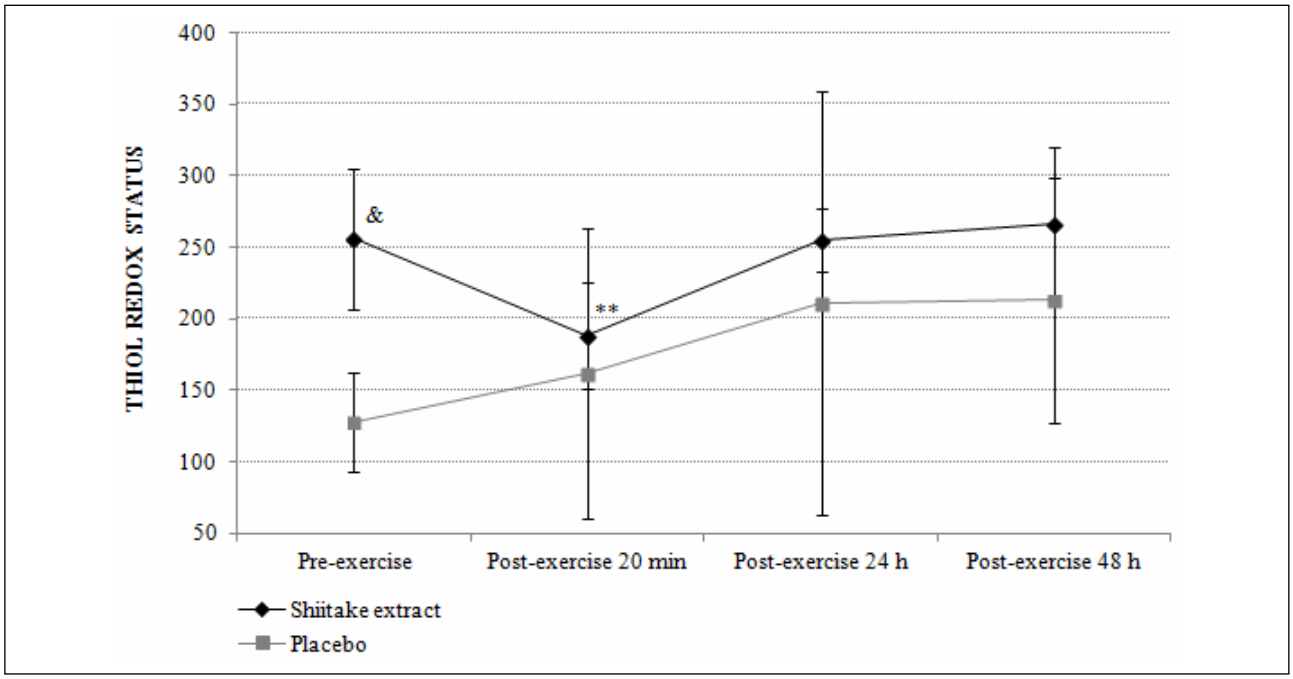

Fig. 1. Changes in blood thiol redox status (GSH_t-2GSSG/GSSG); ** $P<0.01$ indicates significant differences pre-exercise *vs*. post-exercise; $P<0.001$ indicates significant differences shiitake extract *vs.* placebo.

of IL-6 and IL-10 released in response to NO generation during prolonged eccentric exercise.

DISCUSSION

Our study showed that running exercise finished with an eccentric phase caused a considerable damage in skeletal muscle expressed with the high activity of CK and the shift in immune cells, mainly monocytes and neutrophils, which participate in regenerative process and are a source of inflammatory mediators such as H_2O_2, NO, IL-1β, TNF-α, IL-6 and IL-10 within muscle. Shiitake extract did not demonstrate any effect on CK activity and immune cells number at post-exercise period. This indicates that shiitake mushrooms are not useful to enhance the skeletal muscles regeneration after intense exercise.

Moreover, shiitake extract affected H_2O_2 concentration neither before nor after the exercise. According to Hartmann (19), ergothioneine has relatively poor direct reactivity with H_2O_2. However, there have been some reports of ergothioneine and shiitake protective effects in erythrocytes and peripheral blood mononuclear cells (20-22). By contrast, shiitake significantly elevated nitric oxide concentration at pre- and post-exercise period. Misiti *et al*. (23) observed that NO generation is related to ergothioneine-induced decomposition of S-nitrosothiols to NO and thiols. The formation and decomposition of S-nitrosothiols represent mechanism for the storage and transport of NO *in vivo* (24). Lee *et al*. (25) demonstrated that water-soluble fractions obtained from shiitake mushrooms stimulate the functional activation of macrophages including NO production, cytokine expression and phagocytosis. In shiitake group, we observed that the parallel increase in NO, IL-6 and IL-10 expressed the positive correlations. The NO production is an important signalling event in regulation of the expression of cytokines in immune cells and/or contracting skeletal muscles (14).

The high antioxidative properties of shiitake mushrooms were recently confirmed by Reis *et al*. (26) through the measurement of reducing power, free radical scavenging activity and lipid peroxidation inhibition. Our study did not show significant antioxidative activity of shiitake extract in relation to lipid peroxidation. Yet, shiitake prevented a post-exercise increase in 8-isoprostanes' concentration.

Shiitake extract revealed its antioxidative activity through two-fold reduction of GSSG level and increase of thiol redox status which constitutes the cell's reducing power. Kappusamy *et al*. (21) and Kawano *et al*. (27) suggested that one of the possible mechanisms *via* which shiitake extract induces the changes in glutathione is the interaction between ergothioneine and glutathione-related enzymes such as glutathione peroxidase and glutathione reductase.

Thiol redox status has been favourably linked to two redox sensitive transcription factors, nuclear factor-κB (NF-κB) and hypoxia-inducible factor-1α (HIF-1α). Expression of these transcription factors may be induced by extremely high or low levels of reduced and oxidized glutathione, resulting in increased cytokine expression (28, 29). Our previous study revealed the strong relation of thiol redox with IL-6, IL-10 and TNF-α (30). Thiol redox microenvironment plays a major role in production of cytokines which participate in reconstruction and remodelling of injured muscles (28, 31, 32).

Shiitake-induced changes in thiol redox increased the IL-10 level but did not induce significant changes in IL-1β, TNF-α and IL-6. Yu *et al*. (33) observed that mushroom extracts alone have no effect on cytokine production but co-stimulation with either lipopolysacharide or ovalbumin induce TNF-α, IFN-γ, and IL-1β. In our study, the shiitake inability to modulate the inflammatory response could be caused by the low amount of ergothioneine in extract. Menghini *et al*. (34) demonstrated an increase of IL-1β and decrease of TNF-α expression only when macrophages were treated with sub-toxic doses of the shiitake extract. According to Markova *et al*. (35) maximal effects require prolonged exposure because of the slow rate of ergothioneine accumulation.

It is difficult to determine how much shiitake extract should be taken by physically active men to induce beneficial effects One gram of dried powder of shiitake contains 1.98±0.11 mg of ergothioneine (6). An average adult consumes approx. 7 mg of ergothioneine per day in the diet. A rat absorption study demonstrated that 90% of the ergothioneine provided in the diet is absorbed (36). If the human absorption capacity is similar to that found in the rat, then the majority of ingested ergothioneine is retained. According Mayumi *et al*. (36), the supplementation on the order of 5–10 mg would be suitable for an average human adult. Recently, Benson *et al*. (37) showed the anti-inflammatory effects of ergothioneine already at a dose of 0.5 mg daily in patients with articular pains. Ergothioneine supplementation significantly reduced the pain and improved the range of motion in specific joints already at 1 week. Residual effects were seen 6 weeks after stopping consumption of the ergothioneine supplement. According Spierings *et al*. (38), most of the patients with liver disease or cancer use 3 g dose of shiitake extract (approx. 6 mg of ergothioneine), and 9 g dose (approx. 12 mg of ergothioneine) does not cause the adverse effects and is well tolerated by 85% of healthy subjects.

Our study is the first concerning the combination of shiitake ingestion with physical exercise in humans. The results show that shiitake extract at a dose of 1400 mg daily for 10 days does not affect the inflammatory response but demonstrates the antioxidant action through the regulation of nitric oxide concentration and thiol redox status. However, potential recommendation of dried shiitake mushrooms and introduction to the athletes' diet as a source of ergothioneine requires further studies.

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Author's address: Prof. Agnieszka Zembron-Lacny, Department of Sports Medicine, Faculty of Physical Culture in Gorzow Wielkopolski, University School of Physical Education in Poznan, 13 Estkowskiego Street, 66-400 Gorzow Wielkopolski, Poland.
E-mail: agzem@gorzow.home.pl