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Effect of aerobic exercise and resveratrol supplementation on antioxidant enzymes and cytokines of skeletal muscle in mice

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Abstract
Background: The aim of this research was to explore how both aerobic exercise and resveratrol supplementation impact the antioxidant enzymes and immune function of skeletal muscle in mice.
Methods: A total of four groups were established, consisting of six-week-old male ICR mice: 1) The CON group (Control, n=7), 2) The RE group (Resveratrol supplementation, n=7), 3) The EX group (Aerobic exercise, n=7), 4) The EX+RE group (Aerobic exercise and resveratrol supplementation, n=7). Aerobic exercise was conducted on a treadmill at a speed of 15 meters per minute (corresponding to 60% of their maximum oxygen consumption), with a 0-degree incline. This exercise regimen was carried out five days a week for a duration of six weeks. Additionally, resveratrol was administered at a dosage of 25mg per kilogram of body weight once a day, five days a week, for a total of six weeks. To assess the levels of specific markers in the gastrocnemius muscle, such as SOD (Superoxide Dismutase), CAT (Catalase), TNF-1 (Tumor Necrosis Factor-1), and IL-6 (Interleukin-6), Western blotting analysis was employed.
Results: The expressions of SOD 1 and 2 were higher in all groups compared with CON (p<0.05 for all). EX and EX+RE were higher than RE (p<0.05). While no difference in SOD 1 was detected between EX and EX+RE, SOD 2 was lower in EX+RE relative to EX (p<0.05). CAT were higher in EX and EX+RE than CON or RE (p<0.05 for all). Interestingly, CAT was greater in EX+RE than EX (p<0.05). The expressions of TNF-1α and IL-6 in all groups were significantly lower than CON group (p<0.05 for all). TNF-1α in EX+RE group was significantly lower than RE and EX groups (p<0.05). IL-6 in EX+RE group was significantly lower than RE and EX groups.
Conclusions: Co-treatment of aerobic exercise and resveratrol supplementation have a positive synergistic effect for antioxidant enzymes and immune function.

Keywords: Aerobic exercise, resveratrol, antioxidant enzyme, cytokine

1. Introduction
Regular aerobic exercise increases the expression of antioxidant enzymes, and has a positive effect on the concentration of antioxidants in the blood and the activity of antioxidant enzymes [1]. Moderate-intensity exercise is known to reduce the risk of cardiovascular diseases that affect blood circulation, improve health, and prevent various diseases [2]. However, energy metabolism is promoted and oxygen consumption in the body is increased, resulting in oxidative stress, which can increase reactive oxygen species and cause an imbalance in the antioxidant defense system during high-intensity exercise [3]. In addition, the human body defends cells by acting on various antioxidant enzymes and defense systems to counter the continuous attack of reactive oxygen species [4, 5].
Recently, various health functional foods that can be expected to be effective in health promotion as well as regular exercise for antioxidant and immune function improvement are attracting attention [5]. Resveratrol (trans-3, 5, 4-trihydroxy-trans-stilbene) is a natural substance that protects against invasion of external microorganisms or UV stimulation [6]. Wine was pointed out as the cause of the 'French paradox', which had a remarkably low disease-related mortality rate, and it was found that the French people consumed a large amount of red wine containing Resveratrol [7, 8]. In particular, resveratrol removes reactive oxygen species by activating antioxidant enzymes such as SOD, MnSOD, Catalse, and GPx [9].

Resveratrol induces an anti-inflammatory response by inhibiting the activity of cyclooxygenase (COX) and 5-lipoxygenase in arthritis, chronic inflammatory bowel disease, Crohn's disease, and chronic inflammatory dermatitis, psoriasis, in terms of immune and inflammatory responses, thereby reducing the expression of inflammatory cytokines [6, 10]. In addition, it induces SIRT1 by activating AMP-activated protein kinase (AMPK), which acts as a sensor for intracellular energy homeostasis, and then activates PGC-1α, which acts as a mitochondrial biosynthesis regulator [8]. And it affects mitochondrial biosynthesis and function improvement through the AMPK-SIRT1-PGC1α pathway, which in turn acts as a mechanism affecting aerobic exercise capacity [11].

Regular aerobic exercise and resveratrol intake have a positive effect on the body's antioxidant function and immune function. To date, previous studies have investigated antioxidant and immune function following exercise or resveratrol single treatment [3, 6]. However, studies comparing the effects of single treatment and combined treatment at the same time are insufficient. Furthermore, most of the studies are blood analysis, and studies on antioxidant and immune defense mechanisms in skeletal muscle tissue are also lacking. The aim of this research was to explore how both aerobic exercise and resveratrol supplementation impact the antioxidant enzymes and immune function of skeletal muscle in mice.

2. Methods

2.1. Laboratory animal

As for the experimental animals in this study, 6-week-old rats (n=28) with the same birth period were distributed from the experimental animal center, and then 7 rats were randomly divided into each 4 groups after going through an adaptation period: Control group (CON, n=7), Aerobic exercise group (EX, n=7), Resveratrol supplementation group (RE, n=7), Aerobic exercise and resveratrol supplementation group (EX+RE, n=7). The growth environment of the experimental animals was maintained at a 12-hour cycle of light and dark, room temperature of 20 °C, and humidity at 40%.

2.2. Aerobic exercise program

The type of aerobic exercise applied to the test subjects was performed with moderate-intensity treadmill running. Animals were adapted to treadmill exercise at an inclination of 0°with 8 to 10 m/min for one week, gradually increasing from 10 to 30 min/day. Treadmill training went on for six weeks, 5 times a week 60 min per session, following this adaptation period. The incline of running belt constantly stayed at zero angle. The treadmill speed was 8 m/min for 10min, then gradually increasing to 15 m/min for 50min, which corresponded to an average VO_2max of 60% [12].

2.3. Resveratrol supplementation oral administration

Resveratrol (Sigma Aldrich Inc.) was administered by dissolving 0.1 ml of Dimethyl Sulfoxide (DMSO) solution (25 mg/d × body weight). For 6 weeks, the solution was orally administered at the same time 5 times a week using a needle for oral administration in mice in a disposable 1ml syringe.

2.4. Intra-skeletal muscle excision

After all exercise was completed, the experimental animals of each group were intraperitoneally injected with a general anesthetic mixed with Zoletil (Zoletil, Virvac Laboratories), Rompun (Rompun, Bayerkorea) and physiological saline in a ratio of 2:1:2 (1ml/kg). was anesthetized. After that, skeletal muscle gastrocnemius (GM; gastrocnemius) was extracted, immediately frozen in liquid nitrogen, and stored at -80°C until analysis.

2.5. Analysis method

After protein extraction from the excised tissue (GM: Gastrocnemius), electrophoresis (20g sample, 100V, 2 hours) on 10% SDS-Polyacrylamide gel was performed, and then transferred to PVDF membrane (Amersham, USA) (190mM glycine, 50mM). Tris-base, 20% methanol, 0, 05% SDS) and blocking was performed using 5% skim milk. And after washing with PBS solution, primary antibodies SOD 1, SOD 2, CAT, TNF-1, IL-6 (catalogue #: sc-271014, sc-137254, sc-271803, sc-133192, sc-57315, Santacruz biotechnology, USA) and secondary antibody (catalogue #: sc-525409, Santacruz biotechnology, USA) after incubation, the relative intensity of the imaging system reactive bands was quantified using ECL detection reagent solution (catalogue #: RPN2106, Amersham, USA).

2.6. Data Analysis

For the measured data, the mean and standard deviation were calculated using the SPSS Win 25.0 statistical program, and one-way ANOVA was applied to verify the mean difference between groups. The acceptance criterion of the hypothesis was set as $p<0.05$. The data in the graph was expressed as mean±SE, and if there was a statistically significant difference in the analysis results, post-hoc verification was performed using Tukey's post hoc analysis.

3. Results

3.1. Changes in SOD

The results of analysis of SOD changes among the aerobic exercise group and resveratrol supplementation group (EX+RE), aerobic exercise group (EX), resveratrol supplementation group (RE), and control group (CON) following 6 weeks with aerobic exercise were shown. The EX, RE, and EX+RE groups were showed a statistically significantly higher SOD than the CON ($p<0.05$), but in the case of SOD 1, there was no statistically significant difference between the EX+RE and EX. However, the expression of SOD 2 was significantly higher in the EX than the EX+RE ($p<0.05$) (Figure 1).

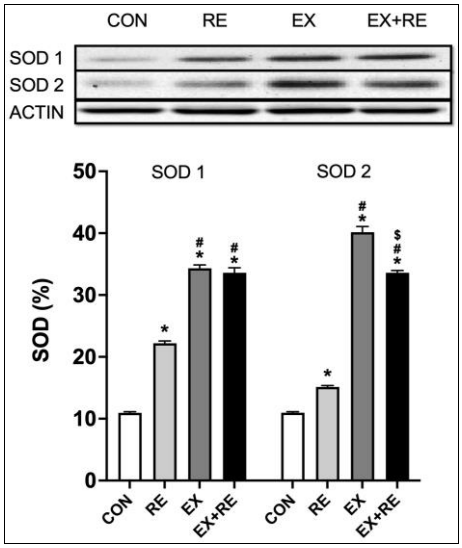

Fig 1: The effect of aerobic exercise and resveratrol supplementation on SOD in mice skeletal muscles; Data is shown as mean±SE (n=28); $^{*}p<0.05$ vs. CON, $^{\#}p<0.05$ vs. RE, $^{\$}p<0.05$ vs. EX.

3.2. Changes in CAT

The results of analysis of CAT changes among the aerobic exercise group and resveratrol supplementation group (EX+RE), aerobic exercise group (EX), resveratrol supplementation group (RE), and control group (CON) following 6 weeks with aerobic exercise were shown. The expression of CAT was significantly higher in the EX and EX+RE than the CON (p<0.05), and in the comparison between the single treatment groups, the expression level was significantly higher in the EX than the RE (p<0.05) (Figure 2).

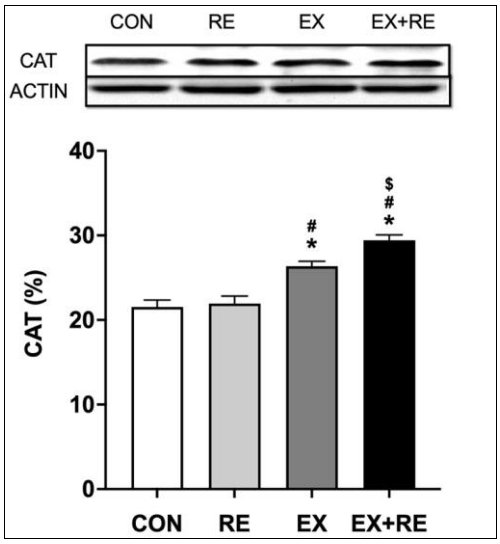

Fig 2: The effect of aerobic exercise and resveratrol supplementation on CAT in mice skeletal muscles; Data is shown as mean±SE (n=28); $^{*}p$<0.05 vs. CON, $^{\#}p$<0.05 vs. RE, $^{\$}p$<0.05 vs. EX.

3.3. Changes in TNF-a

The results of analysis of TNF-α changes among the aerobic exercise group and resveratrol supplementation group (EX+RE), aerobic exercise group (EX), resveratrol supplementation group (RE), and control group (CON) following 6 weeks with aerobic exercise were shown. The expression of TNF-α was statistically significantly lower in the comparison between the CON and the single treatment groups with EX+RE (p<0.05). Also, in comparison between single treatments, the EX expressed higher TNF-α than the RE, but there was no statistically significant difference (Figure 3).

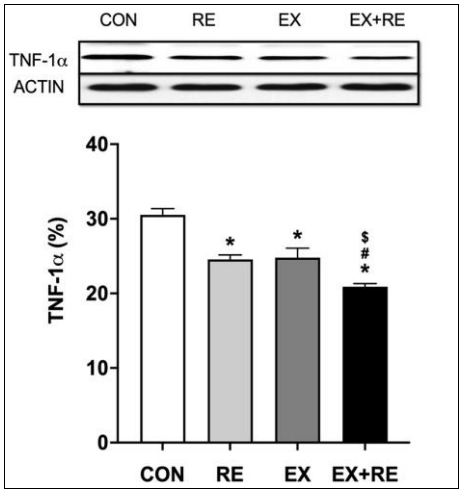

Fig 3: The effect of aerobic exercise and resveratrol supplementation on TNF-α in mice skeletal muscles; Data is shown as mean±SE (n=28); $^{*}p$<0.05 vs. CON, $^{\#}p$<0.05 vs. RE, $^{\$}p$<0.05 vs. EX.

3.4. Changes in IL-6

The results of analysis of IL-6 changes among the aerobic exercise group and resveratrol supplementation group (EX+RE), aerobic exercise group (EX), resveratrol supplementation group (RE), and control group (CON) following 6 weeks with aerobic exercise were shown. The expression of IL-6 was statistically significantly lower in the EX+RE compared to the CON with the EX and RE (p<0.05). Also, in comparison between single treatments, the EX expressed higher IL-6 than the RE, and showed a statistically significant difference (p<0.05) (Figure 4).

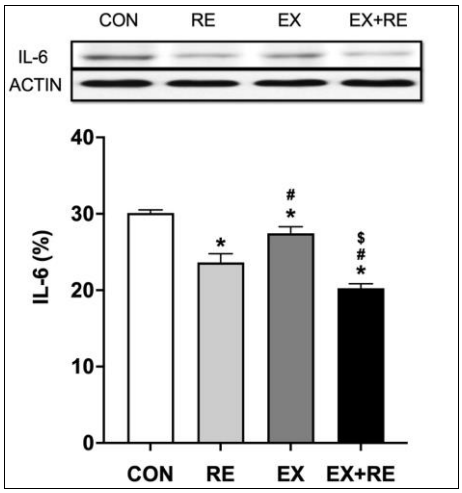

Fig 4: The effect of aerobic exercise and resveratrol supplementation on IL-6 in mice skeletal muscles; Data is shown as mean±SE (n=28); $^{*}p$<0.05 vs. CON, $^{\#}p$<0.05 vs. RE, $^{\$}p$<0.05 vs. EX.

4. Discussion

The effects of aerobic exercise and resveratrol intake for 6 weeks on antioxidant enzymes (SOD, CAT) and cytokines (TNF-α, IL-6) in skeletal muscle of white rats were analyzed. The antioxidant enzymes SOD and CAT in skeletal muscle were significantly increased in all groups compared to the control group (CON), and in comparison with the single treatment group, the aerobic exercise group (EX) significantly increased than the resveratrol supplementation group (RE). In the case of SOD 1, there was no significant difference between the aerobic exercise group and aerobic exercise and resveratrol supplementation group (EX+RE), but the expression of CAT was higher in the EX+RE than in the EX.

In the previous studies on the relationship between aerobic exercise and antioxidant enzymes, it was reported that endurance exercise using a treadmill for 9 weeks increased SOD activity [13, 14], and was reported that the activity of SOD increased as it went up [15]. In addition, it was reported that SOD, CAT, and GPX activities were changed in liver and heart as a result of swimming training with animals [16, 17]. According to a study by Harris M.B *et al*., SOD and CAT concentrations increased according to 12 weeks of endurance training, and reported that GPX activity was increased [14]. This is consistent with the results of this study as both RE and EX had positive results on the expression of antioxidant enzymes. In addition, it has been reported that oral administration of resveratrol reduces the expression of reactive oxygen species, NADPH oxidase 4, activates the expression levels of SIRT1, SOD2, and GPx [18], and activates SOD, MnSOD, Catalase, and GPx to remove ROS [9].

In this study, TNF-1α and IL-6 decreased in the RE compared to the control group, and decreased the most in the EX+RE. These results are consistent with the results of the study that

resveratrol inhibited COX-2 and intracellular calcium in addition to the production and expression of inflammatory cytokines TNF-α, IL-6, and IL-8 in HMC-1 cells [6]. In addition, resveratrol reduces the expression of inflammatory cytokines (TNF-α, IL-1, IL-6) as an anti-inflammatory effect, and improves microcirculation and regulates apoptosis by inhibiting the destruction of the vascular endothelial cell wall and leukocyte adhesion were consistent with the results of the study [6]. In particular, resveratrol has been shown to reduce oxidative stress, vascular inflammation, smooth muscle hypertrophy, cardiac hypertrophy, and cardiac ischemia damage by regulating various molecules related to the cardiovascular system [8].

Damage and neutrophil infiltration were reduced, and IL-6, MCP-1, and COX-2 expression levels were decreased to alleviate oxidative damage and stimulate apoptosis [19]. Resveratrol contains a chalcone compound, and chalcone reduces IL- 6, It was also consistent with the results of the study that reduced the secretion of TNF-1α and IL-8 [20]. Meanwhile, inflammatory cytokines TNF-1α and IL-6 were decreased in the EX and the EX+RE compared to the control group. These results showed that the expression of TNF-α and IL-6 in skeletal muscle of rats was significantly reduced in the low-intensity and high-intensity exercise group than in the control group with diabetes [13] and in rats after 4 weeks of high-intensity exercise. This is consistent with the study results showing a significant increase in the mRNA expression level of TNF-1α in gastrocnemius muscle compared to the control group [21].

Therefore, both the RE and the EX suppressed the inflammatory response compared to the control group through this study, and the EX+RE showed the most effective inhibition of the inflammatory response. However, no synergistic effect was observed in the EX+RE on the expression of cytoplasmic SOD 1 and mitochondrial SOD 2 in this study. In the case of CAT, since an additional increase in expression was observed in the EX+RE, the additional decrease in cytokines in the EX+RE identified in this study is thought to be due to overexpression of CAT.

5. Conclusions

The expression of antioxidant enzymes increased in SOD and CAT in all groups of the single treatment group and the combined treatment group, and it was found that it had a positive effect on improving immune function by reducing inflammatory cytokines TNF-α and IL-6.

6. Conflict of Interest

None declared.

7. Funding Sources

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