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Original Article

Effect of a Six Week-Swimming Interval Training with Resveratrol Consumption on Apoptotic Markers in the Liver Tissue of Aged Rat

Maryam Mehboodi[1], Habib Asgharpour[*1], Seyed Ali Hosseini[2], Reza Rezaeeshirazi[1]

1. Department of Physical Education and Sport Sciences, Aliabad Katoul Branch, Islamic Azad University, Aliabad Katoul, Iran
2. Department of Sport Physiology, Marvdasht Branch, Islamic Azad University, Marvdasht, Iran

[*] **Corresponding Author:** Department of Physical Education and Sport Sciences, Aliabad Katoul Branch, Islamic Azad University, Aliabad Katoul, Iran. **Tel:** +989113922124**, Email address**: habibasgharpour@gmail.com

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Introduction: Aging involves a decrease in physiological function, physical activity and nutrition that modulate body functions. The aim of this study was to evaluate the effect of a six week-swimming interval training with resveratrol consumption on apoptotic markers in the liver tissue of aged rat.

Methods: In this experimental study, thirty-two 20-month aged rats weighing 350-370 g were divided into four groups of 8 rats including 1) sham, 2) training, 3) resveratrol and 4) training + resveratrol. For six weeks, groups 3 and 4 received 100 mg/kg of resveratrol supplementation dissolved in 1% methylcellulose daily by gavage, and groups 2 and 4 performed swimming training three times a week. One-way analysis of variance with Tukey's post- hoc test was used to analyze the data ($p < 0.05$).

Results: Bcl2 Gene expression levels in the resveratrol and the exercise + resveratrol groups were significantly higher than the sham and exercise groups ($p < 0.05$). Bax levels in the exercise + resveratrol group were lower than the resveratrol group, and the levels in the resveratrol group were higher than the sham group($p <0.05$).also Bax/Bcl2 levels in the exercise + resveratrol group were significantly lower than the exercise group($p < 0.05$).

Conclusion: It seems that swimming interval training with resveratrol consumption has beneficial effects on anti-apoptotic markers, however, the effect of swimming interval training on liver apoptosis in the aging is still unknown and more studies are needed in this field.

Keywords: Swimming Training, Resveratrol, Apoptosis, Liver, Aging

Introduction

Exercise plays a role in inhibiting apoptosis, inflammation and liver fibrosis by improving the antioxidant system, inhibiting caspases and Bax (1). However, the effect of exercise depends on the type and intensity, and different results have been reported following high-intensity interval training (HIIT). For example, HIIT increased levels of superoxide dismutase (SOD), catalase, and decreased malondialdehyde (MDA) in the liver tissue of doxorubicin-poisoned rats (2); on the other hand, HIIT increased 8-oxoguanine DNA glycosylase (OGG1) but had no effect on 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the liver tissue of rats (3). HIIT training also increased Bax and caspase-3 gene expression levels in the liver tissue of aged female rats (4).

Due to the contradictory results related to the effect of HIIT on oxidative stress and apoptosis markers in liver tissue, researchers have recently drawn attention to the use of herbs along with high intensity exercise, as medicinal plants have less side effects than synthetic drugs and on the other hand

have more antioxidant effects (4). Resveratrol with its antioxidant and anti-inflammatory properties is used in the treatment of many liver and cardiovascular diseases (5). In this regard, consumption of 50 mg/kg silymarin and 10 mg resveratrol increased antioxidants and decreased liver enzymes and inflammatory factors in the liver tissue of thioacetamide-poisoned rats (5).

Consumption of resveratrol increased total antioxidant capacity and decreased MDA in the liver tissue of rats with polycystic ovary syndrome (6); Resveratrol also decreased aspartate aminotransferase (AST), alanine aminotransferase (ALT) and caspase-3 in the liver tissue of alcoholic fatty liver rats (7). Despite many investigations, no study has been found to evaluate the anti-apoptotic effect of resveratrol supplementation along with HIIT. Considering the need of the elderly to do sports activities and their satisfaction of the benefits of sports, it seems that the present study could provide further information on the interactive effect of HIIT and resveratrol consumption in the liver tissue of aged rat. Therefore, the present study was performed to investigate the effect of six weeks of high intensity interval training along with resveratrol consumption on some hepatic apoptosis markers of aged rat.

Methods

In this experimental study, thirty-two 20-month aged rats weighing 350-370g, were purchased and transferred to the laboratory. The rats were kept in the laboratory for one week to adapt to the new environment and then were randomly divided into four groups: 1) sham, 2) training, 3) resveratrol and 4) training + resveratrol. For six weeks, groups 3 and 4 received 100 mg/kg of 1% methylcellulose-soluble resveratrol supplement daily by gavage (8) and groups 2 and 4 performed swimming training three times a week. In this study, the swimming training protocol was based on Terada et al.'s study protocol (9). The swimming training protocol consisted of 14 sets of 20 seconds of swimming and 10 seconds of rest between sets. This protocol was performed for six weeks and three days a week. The initial load was 9% of the rats' body weight, to which 1% was added each week, so that in the last week the rats exercised at 15% of their body weight. At the end of 48 hours after the last training session and resveratrol consumption, fasting rats were anesthetized with ketamine and xylazine. After extraction of their liver tissue, all tissues were placed in a nitrogen tank and transferred to the laboratory.

Bax and Bcl2 gene expression levels were measured by real-time PCR. The primer sequence of the research variables is reported in Table 1. To analyze the research findings, the Shapiro-Wilk test and one-way analysis of variance with Tukey's post- hoc test were used ($p < 0.05$).

Ethical consideration

The present study with the code of ethics IR.IAU.M.REC.1399.036 was approved by the Research Ethics Committee of the Islamic Azad University of Marvdasht.

Results

Bax, Bcl2 and Bax/Bcl2 gene expression levels are shown in Figures 1-3, respectively.

The results of analysis showed that Bax gene expression levels in the resveratrol group were significantly higher than the sham group ($p = 0.01$, MD = -0.630). However, in the training + resveratrol group, the levels were significantly lower than the resveratrol group ($p = 0.01$, MD = 0.063) (Figure 1).

Bcl2 gene expression levels in the resveratrol ($p = 0.02$, MD = 0.497) and training + resveratrol ($p = 0.003$, MD = 0.675) groups were significantly higher than the sham group. Also in the resveratrol ($p = 0.002$, MD = 0.715) and training + resveratrol ($p = 0.001$, MD = 0.892) groups, the Bcl2 levels were significantly higher than the training group (Figure 2).

Also Bax/Bcl2 ratio in the training + resveratrol group were significantly lower than the training group ($p = 0.029$, MD = 1.476) (Figure 3).

Discussion

The results of the present study showed that swimming training had no significant effect on Bcl2 and Bax gene expression levels in the liver tissue of aged rat. Despite the beneficial effects of exercise on improving quality of life, prevention of diseases and improving metabolism, studies show that exercise with the mechanism of phospholipase A2 (PLA2), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase increases ROS during strenuous exercise. Normally, the antioxidant system neutralizes ROS. However, the balance of oxidative-antioxidant stress depends on several factors such as age, sex, type and intensity of physical activity, so that anaerobic physical activity and even close to the anaerobic threshold, increases lipid peroxidation and disrupts the enzymes of the electron transfer chain complex; this leads to an increase in inflammatory factors, increase in cytochrome P450-dependent oxygenase, decrease in the efficiency of the antioxidant system, oxidative damage to the nucleus and cell membrane, activation of caspases and the onset of cell death (10, 11). In support of the findings of this study on apoptotic markers, four weeks of HIIT had no significant effect on reducing Bax and Bax/Bcl-2 ratio in the heart tissue of elderly C57BL/6 mice, however, these researchers reported increased Bcl2 gene expression in young and elderly trained mice compared to their control groups (11); in addition, Ammar et al. showed that aerobic, anaerobic and combined exercises increased plasma oxidative stress in non-athlete men (10); also, 12 weeks of HIIT with 85 to 90% Vo2max

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had no significant effect on Bax and caspase 3 gene expression levels in rats but increased Bcl2 levels and Bcl-2/Bax ratio (12). HIIT and moderate-intensity continuous training improved the lipid profile of diabetic patients with non-alcoholic fatty liver, but had no significant effect on their liver damage indices (13). Therefore, it seems that the effect of HIIT on the pathways of oxidative-antioxidant stress and apoptosis depends on the type of training, training intensity, number of training sessions and age of the subjects and is not yet well known.

Table 1. Primer sequence of research variables

Genes	Primer Sequences	Sizes (bp)
Bax	Forward: 5'- CTGCAGAGGATGATTGCTGA -3' Reverse: 5'- GATCAGCTCGGGCACTTTAG-3'	174
Bcl2	Forward: 5'- ATCGCTCTGTGGATGACTGAGTAC-3' Reverse: 5'- AGAGACAGCCAGGAGAAATCAAAC-3'	134
B2m	Forward: 5'- CGTGCTTGCCATTCAGAAA -3' Reverse: 5'-ATATACATCGGTCTCGGTGG -3'	244

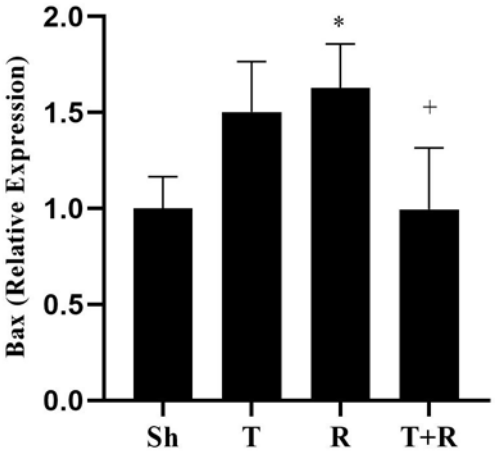

Figure 1. Bax gene expression levels in the four research groups
Sham (Sh), Training (T), Resveratrol (R), Training + Resveratrol (T + R)
***p < 0.05 Significant increase in R group compared to the sham group.**
+p < 0.05 Significant decrease in T + R group compared to the resveratrol group

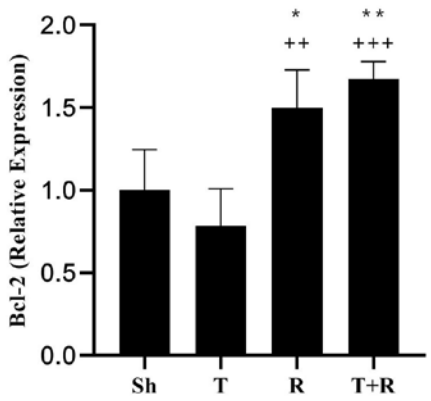

Figure 2. Bcl2 gene expression levels in the four research groups
Sham (Sh), Training (T), Resveratrol (R), Training + Resveratrol (T + R)
****p < 0.01 and * p < 0.05 Significant increase in R and T+R groups compared to the sham group.**
+++p < 0.001 and ++ p < 0.01 Significant increase in R and T+R groups compared to the training group

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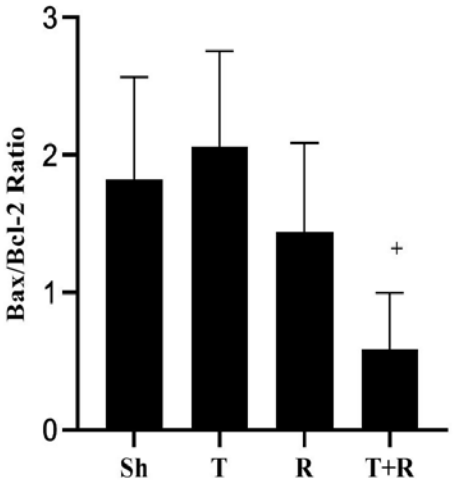

Figure 3. Bax/Bcl2 levels in the four research groups
Sham (Sh), Training (T), Resveratrol (R), Training + Resveratrol (T + R)
+ $p < 0.05$ Significant decrease in T + R group compared to the training group

The results of the present study showed that resveratrol consumption increased Bax and Bcl2 gene expression in the liver tissue of aged rat. Resveratrol with the formula 3,4', 5 trihydoxystilbene is a natural polyphenol found in most plants; the beneficial effects of this herb in reducing oxidative stress, increasing antioxidants, reducing inflammatory factors, and improving heart function and atherosclerosis have been reported. This polyphenol with its antioxidant effects inhibits induced tumors by cyclooxygenase-2 (COX-2) and inhibits the function of NF-Kb by increasing the activity of nitric oxide synthetase; also, by increasing the expression of nuclear respiratory factor-2 (Nrf2) reduces homoxygenase-1 and activates the anti-apoptotic pathway of Bcl2 through the c-Jun N-terminal kinase (JNK) pathway (14); however, the effects of this supplement are dose-dependent, so that low doses have little effect on improving cell function and very high doses cause poisoning, but resveratrol consumption is dependent on age and baseline levels of cell dysfunction, and 2.5, 25 and 100 mg/kg have been reported to be the lowest to the most effective doses, respectively, while the most optimal dose for cardiovascular protection was reported to be 100 mg/kg (14,15). In this regard, the researchers showed that 24 and 72 hours after taking resveratrol, levels of oxidative stress and apoptotic induction factors decreased and angiogenic factors increased (16); The use of 50 and 10 μM resveratrol reduced H2O2-induced oxidative stress in C2C12 cells and increased the activity of SIRT1 and Nrf2 and decreased ROS and oxygenase-1. In addition, the results of this study showed that higher doses had more favorable effects (17). Due to the different conditions of resveratrol use, it seems that the baseline levels of oxidative stress, dosage, duration of use and drug interventions impact the effectiveness of this supplement and there is no complete information in this regard, and hence according to Shaito et al.'s study, the evaluation of effective dose of this supplement has to be investigated.

The results of the present study also showed that swimming training along with resveratrol increased Bcl2 gene expression levels and decreased Bax/Bcl2 levels compared to the HIIT group. According to previous studies, HIIT appears to increase hepatic apoptosis by increasing oxidative stress, inflammatory factors, activation of cell membrane and mitochondrial damage pathways (10,11), while resveratrol supplementation with its antioxidant, anti-inflammatory and anti-apoptotic effects inhibits the process of hepatic apoptosis following HIIT, especially in the elderly (14). Regarding the interactive effect of training and resveratrol supplementation, Mehri et al.'s study showed that aerobic training and 50 mg/kg resveratrol supplementation had a synergistic effect on reducing Bax levels, but either of these two interventions increased Bcl2 (18). However, in another study, resveratrol and exercise increased Sirt1 gene expression and decreased apoptosis-inducing proteins. Also, these two interventions interactively improved liver enzymes in rats with non-alcoholic fatty liver (19). Due to the relative increase in Bax gene expression levels following two interventions, it seems that aging conditions and apoptosis are the main factors for which there is no exact prevention and treatment. Nonetheless, high intensity training intervention in these situations is still challenging. The important finding of this study was that in addition to the increase in Bcl2 in the training + resveratrol group, Bax/Bcl2 ratio decreased compared to the training group, so it seems that resveratrol supplementation is relatively able to improve aging-induced apoptosis.

Conclusion

Swimming training along with resveratrol appears to exert anti-apoptotic effect in liver tissue of aged animals.

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Study limitations

Due to the complexity of the cell death pathway, it seems that the lack of measurement of further markers is one of the limitations of the present study. Therefore, it is suggested that in future studies, stress-oxidative-antioxidant apoptotic markers of external and mitochondrial pathways be evaluated. In addition, it seems that the lack of examination of tissue pathology is another limitation of the present study, which suggests that future studies should be considered by researchers.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

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Authors' contributions

Design and conceptualization: Habib Asgharpour, Reza Rezaeeshirazi; Methodology: Maryam Mehboudi; Data analysis: Seyed Ali Hosseini; Supervision: Habib Asgharpour, Seyed Ali Hosseini, Reza Rezaeeshirazi.

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