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Original Article

The Impact of Eight Weeks of Interval Training and Resveratrol Supplementation on Autophagy-Related Factors in Rat Cardiac Tissue

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Abstract

Background and aims: Autophagy plays a critical role in maintaining cellular balance under normal physiological conditions. This study aimed to evaluate the influence of eight weeks of high-intensity interval training (HIIT) combined with resveratrol intake on the cardiac expression levels of Kelch-like ECH-associated protein 1 (Keap1) and nuclear factor erythroid 2-related factor 2 (Nrf2) proteins in rats.

Methods: The experimental design employed a post-test-only approach with a control group. Forty male Wistar rats aged 12–18 months were randomly divided into resveratrol, HIIT, control, and combined HIIT with resveratrol groups (n=10 per group). The exercise and supplementation interventions were conducted over eight weeks. One-way ANOVA was performed to evaluate differences among groups, followed by the Tukey post-hoc test to determine the specific sources of these differences.

Results: Significantly higher levels of NRF2 were observed in the combined training and resveratrol group compared to the control group ($P=0.001$). The groups undergoing only HIIT or only resveratrol supplementation also showed elevated NRF2 expression in comparison to the control group ($P=0.001$ for both). Correspondingly, Keap1 protein levels were significantly reduced in the combined intervention group compared to controls ($P=0.001$), with similar reductions observed in the individual HIIT and resveratrol groups ($P=0.001$ and $P=0.007$, respectively).

Conclusion: Considering that KEAP1 is an inhibitor of NRF2, our findings revealed that the amount of KEAP1 decreased following training and resveratrol consumption. Interval training and resveratrol consumption increased NRF2 by inhibiting the KEAP1 protein.

Keywords: Autophagy, Interval training, Resveratrol

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Introduction

Autophagy is a highly conserved and regulated process, controlled by a series of autophagy-related genes (1). It has a vital role in preserving cellular homeostasis in normal physiological conditions. Autophagy also serves as a defense mechanism against stress. It achieves this by eliminating misfolded proteins and damaged organelles (e.g., mitochondria) while also supplying nutrients and energy through the degradation of cellular components (2). Multiple signaling pathways contribute to the regulation of autophagy. Kelch-like ECH-associated protein 1 (Keap1), a protein abundant in cysteine residues, functions as a negative regulator of nuclear factor erythroid 2-related factor 2 (Nrf2) activity. Within the cytoplasm, Nrf2 associates with Keap1. The Keap1/Nrf2 signaling axis is crucial for maintaining cellular homeostasis under oxidative stress (OS) conditions. Sestrin2, which becomes activated in response to OS, enhances the expression of sulfiredoxin by facilitating the activation of Nrf2. Additionally, Sestrin2 can stimulate Nrf2 by inducing the autophagic degradation of Keap1 in a p62-dependent manner. As a result, Sestrin2 reduces the accumulation of reactive oxygen species (ROS) while enhancing antioxidant defenses by modulating the Keap1/Nrf2 pathway (3).

Engaging in exercise to modulate autophagy has been shown to have positive effects. Impaired autophagy and alterations in its activity contribute to the development of various diseases. Physical activity supports the maintenance of protein homeostasis and enhances the removal of reactive aldehydes. Moreover, elevating the basal level of cardiac autophagy has been reported to strengthen the heart's resilience against future ischemic events. In this context, high-intensity interval training (HIIT), which alternates brief bouts of vigorous exercise with periods of lower-intensity recovery, offers an efficient approach to optimizing health outcomes within a limited timeframe. Research indicates that HIIT may enhance cellular function by facilitating the clearance of defective proteins and organelles via the stimulation of autophagy (4). Since the Keap1/SES2 signaling pathway plays a crucial role in autophagy and improving heart function, no precise results have been reported on the effects of interval training, particularly in middle-aged and elderly samples. Further research is, therefore, needed in this

regard. It appears that, alongside exercise training, the use of herbal supplements is also essential.

Resveratrol (3,4′,5-trihydroxy-trans-stilbene) is a natural polyphenolic compound predominantly found in the roots of certain plants and edible fruits (e.g., grapes and berries). Emerging evidence suggests that resveratrol may activate the autophagic process, which is believed to play a pivotal role in mediating its biological benefits, including anti-inflammatory responses, induction of apoptosis in tumor cells, and protection against oxidative damage (5). Although multiple research groups have proposed potential pathways and molecular intermediaries responsible for resveratrol-induced autophagy, a definitive mechanistic link has not been established yet. When combined with interval training, the autophagy-enhancing potential of resveratrol may lead to amplified cellular benefits. Nevertheless, scientific literature offers limited insights into how this combination affects cardiac autophagy, particularly via the NRF2/Keap1/Sestrin2 axis. Systematic studies in available databases yielded insufficient evidence to draw firm conclusions in this context. Hence, the present investigation aims to evaluate the impact of an eight-week HIIT protocol, combined with resveratrol administration, on the myocardial expression levels of Sestrin2 and Keap1 in middle-aged rats.

Materials and Methods

An experimental design with a post-test-only approach and a control group framework was implemented in this research. A total of 40 elderly male Wistar rats, aged between 12 months and 18 months, were obtained from the Pasteur Institute to serve as the subjects of this investigation. The animals were housed in standardized laboratory conditions—eight rats per cage—with regulated temperature (25 ± 2°C), a 12-hour alternating light-dark cycle, and unrestricted access to food and water. All housing procedures were performed in alignment with the ethical protocols of the Iranian Society for the Protection of Laboratory Animals.

Following a one-week adaptation period to their new environment, the rats were allocated to four experimental groups through a straightforward randomization process. They included (1) a group receiving only resveratrol supplementation (n = 10), (2) a group subjected solely to HIIT (n = 10), (3) a non-intervention control group (n = 10), and (4) a combined intervention group receiving both resveratrol and HIIT (n = 10). Random assignment to groups was performed using a blinded method involving randomly selected, sealed, opaque envelopes labelled with group identifiers, ensuring an equal allocation probability for each animal. Before the start of the training intervention, the rats underwent a treadmill acclimatization process, which included daily sessions of walking and running to reduce stress and familiarize them with the apparatus. Following a one-week familiarization period, a graded exercise test was conducted to determine the animals' maximum running speed, which was subsequently used to calibrate the intensity of the training protocol.

Rats in the designated training and combined intervention groups participated in an eight-week regimen of high-intensity interval exercise performed on a motorized treadmill. Forty-eight hours after the final exercise session, and following a 12-hour overnight fast, all animals were anesthetized to collect heart muscle tissue samples. Throughout the experimental period, a standard laboratory diet was provided without restriction.

All procedures strictly adhered to national ethical standards governing the use of laboratory animals and were officially approved by the Ethics Committee of Islamic Azad University, Tehran. The research activities were conducted at the Central Tehran Branch of Islamic Azad University in early 2025. Notably, no deaths occurred during the protocol's duration, and all animals completed the study successfully.

The High-Intensity Interval Training Protocol

The HIIT regimen was performed over eight weeks, with participants engaging in five treadmill sessions per week at a 0-degree incline. Each session comprised 5–8 intervals, each lasting 2 minutes, conducted at an intensity corresponding to 80–100% of maximal oxygen uptake. These intervals were interspersed with 2-minute periods of active recovery at 50% of maximal oxygen uptake. The running speed progressed from 22 meters per minute during the initial week to 31 meters per minute by the final two weeks. The number of intervals was increased from 5 in the first week to 8 by the fourth week and maintained thereafter. Each training session also included a 10-minute low-intensity warm-up before exercise and a 5-minute cool-down following completion (6).

Resveratrol Consumption

The resveratrol supplement was administered following established protocols from prior studies. For each rat, 100 µL of either 7% ethanol or 10% dimethyl sulfoxide diluted with water was prepared as a vehicle, into which resveratrol was suspended before administration. To minimize variability among subjects, the solution was prepared in a single batch and intraperitoneally administered to both the supplement and supplement/exercise groups at a dosage of 25 mg/kg body weight over eight weeks (7).

Tissue Method

In this procedure, 48 hours following the final intervention, all rats underwent an 8–10-hour fasting period and were weighed before tissue collection. Anesthesia was administered using a mixture of 10% ketamine and 2% xylazine, at dosages of 100 mg/kg for ketamine and 10 mg/kg for xylazine.

Western Blot Test

Tissue/Cell Lysis

For this purpose, 500 mg of tissue/10^6 cells were homogenized/lysed in 500 µL of lysis buffer (Tables 1 and

Table 1. Results of the One-Way ANOVA Test

Markers	Control	Supplement	Training	Training+Training	F	*P* value
NRF2	1.01±0.05	1.42±0.09	2±0.08	2.47±0.08	233.25	0.001
Keap1	1.02±0.05	1.72±0.09	1.52±0.09	1.52±0.7	83.16	0.001

Note. NRF2: Nuclear factor erythroid 2-related factor 2; Keap1: Kelch-like ECH-associated protein 1.

Table 2. Results of Tukey's Post Hoc Test

Markers	Groups	Mean Difference	Standard Deviation	*P* value
NRF2				
Control	Supplement	0.4	0.5	0.001
	Training	0.98	0.5	0.001
	Training+Training	1.45	0.5	0.001
Training	Supplement	0.57	0.5	0.001
	Training+Training	0.47	0.5	0.001
Supplement	Training+Training	1.05	0.5	0.001
Keap1				
Control	Supplement	0.17	0.5	0.007
	Training	0.37	0.5	0.001
	Training+Training	0.87	0.5	0.001
Training	Supplement	0.2	0.5	0.32
	Training+Training	0.5	0.5	0.001
Supplement	Training+Training	0.7	0.5	0.001

Note. NRF2: Nuclear factor erythroid 2-related factor 2; Keap1: Kelch-like ECH-associated protein 1.

2). Then, the samples were centrifuged in an Eppendorf 5415 R model centrifuge at 4°C and 12,000 rpm for 10 minutes. The clear liquid (the supernatant) containing the extracted protein was collected and stored in a freezer at -20°C.

The normality of the data distribution was examined using the Shapiro-Wilk test. After confirming normality, one-way analysis of variance (ANOVA) was conducted to compare group differences, and Tukey's post-hoc test was applied to identify pairwise differences. All statistical analyses were performed using SPSS (version 26), considering *P*-values ≤ 0.05 as statistically significant.

Results

The one-way ANOVA test showed a statistically significant difference in NRF2 expression among the experimental groups ($P=0.001$). Subsequent post hoc comparisons indicated that rats receiving both HIIT and resveratrol supplementation had the highest NRF2 levels, which were markedly greater than those observed in the control group ($P=0.001$). Likewise, the exercise-only group demonstrated significantly elevated NRF2 expression in comparison with controls ($P=0.001$). Additionally, rats in the resveratrol-only group exhibited a significant increase in NRF2 levels compared to the control group ($P=0.001$). Further analysis revealed that NRF2 expression in the combined intervention group was significantly higher than in the supplement-only group ($P=0.001$) and the exercise-only group ($P=0.001$). Moreover, the exercise group also represented significantly higher NRF2 levels in comparison to the supplement group ($P=0.001$). These differences are detailed in Tables 1 and 2.

Statistical analysis via one-way ANOVA confirmed significant variations in Keap1 protein levels across the experimental groups ($P=0.001$). According to the results of the post hoc comparisons, animals in the combined HIIT and resveratrol supplementation group showed significantly lower Keap1 expression when compared to the control group ($P=0.001$). A similar reduction in Keap1 levels was found in the training-only group relative to controls ($P=0.001$). Similarly, rats receiving only resveratrol supplementation demonstrated a statistically significant difference from the control group ($P=0.007$). Further pairwise comparisons revealed that Keap1 expression in the combined group was significantly lower than in the resveratrol-only group ($P=0.001$). A notable difference was also identified between the exercise-only and resveratrol-only groups ($P=0.032$). Detailed numerical values and statistical comparisons are presented in Tables 1 and 2.

Discussion

The results demonstrated that NRF2 expression was significantly elevated in the group subjected to both exercise and resveratrol supplementation when compared to the control group. An increase in NRF2 levels was also evident in the group receiving exercise alone relative to controls. Likewise, the resveratrol-only group exhibited a notable rise in NRF2 compared to the control group. Furthermore, comparative analysis showed that the combined intervention group had significantly higher NRF2 expression than the resveratrol-only group. A similar trend was observed when comparing the exercise group to the supplement group, and a statistically significant distinction was also found between the combined intervention group and the group undergoing exercise alone.

Regarding Keap1 protein levels, the exercise-plus-resveratrol group displayed a significant increase compared to the control group. The exercise-only group also represented elevated Keap1 levels in comparison to the control group. Additionally, the resveratrol group demonstrated an essential difference from the control group in Keap1 expression. Moreover, the combined group differed significantly from the resveratrol-only group, and a comparable difference was detected between the exercise and resveratrol groups.

In this context, Safdar et al conducted biopsies of the vastus lateralis muscle and found that maintaining an active lifestyle in older adults helps preserve the redox

balance within skeletal muscle cells by stimulating the Nrf2-Keap1 signaling pathway (8). MacNeil et al examined the vastus lateralis muscle in young participants both before and three hours after eccentric exercise, observing a 40.1% increase in nuclear Nrf2 levels but a 35.5% reduction in Keap1 content (9). Gounder et al performed acute and chronic exercise studies in young and old mice, concluding that the Nrf2/antioxidant response element system is activated in young mice after both forms of exercise (10). Nonetheless, a decrease in Nrf2 nuclear translocation and the disruption of antioxidant response element signaling were detected following acute exercise. In contrast, chronic exercise led to the stabilization of Nrf2 in aged mice. Long-term exercise may serve as a protective mechanism against OS associated with aging.

In another experimental investigation involving older Nrf2-deficient mice subjected to treadmill running for 60 minutes daily over two consecutive days, acute exercise was found to elevate ROS levels while decreasing glutathione concentrations in cardiac tissue. This observation suggests that the absence of Nrf2 is correlated with diminished antioxidant defenses. Furthermore, the activation of the Nrf2/ARE pathway was noted in myocardial tissue following acute exercise. The study proposes that acute physical activity can enhance mitochondrial ROS generation within the myocardium. Consequently, ROS appear to play a crucial role in activating Nrf2, as they facilitate the dissociation of Nrf2 from its Keap1 complex (11). In general, a single intense exercise session decreases Nrf2 due to increased OS. However, over time, through several weeks of exercise training, OS is reduced, but Nrf2 levels increase. Given that training in our study led to an increase in Nrf2, since it lasted for several weeks.

Alavi et al concluded that resveratrol, a natural polyphenolic compound, can initiate the activation of the transcription factor Nrf2. Once activated, Nrf2 translocates into the nucleus, where it promotes the transcription of genes responsible for the body's antioxidant defense mechanisms (12). Alavi et al demonstrated that resveratrol modulates oxidative damage and inflammatory signaling via the stimulation of the Nrf2/ARE cascade in a murine model subjected to ischemia/reperfusion injury. Cardioprotective effects were evident through reductions in the myocardial levels of malondialdehyde and myeloperoxidase, as well as decreases in the serum concentrations of lactate dehydrogenase and creatine kinase. Furthermore, treatment with resveratrol led to increased enzymatic activities of antioxidant systems, particularly superoxide dismutase and glutathione peroxidase (13). Kjaer et al investigated the effects of a 14-day protocol involving resveratrol at a dose of 2.5 mg/kg on cardiac tissue in a rat model of coronary artery ligation. In this experiment, resveratrol was co-administered with adult cardiac stem cells marked by enhanced green fluorescent protein. This co-treatment resulted in a notable increase in enhanced green fluorescent protein signal within the peri-infarct area. Evaluation performed seven days after the occlusion of the left anterior descending artery revealed that repeated exposure to resveratrol not only improved cardiac performance but also modulated the cellular microenvironment. These benefits were associated with the upregulated nuclear expression of redox factor-1 and the activation of the Nrf2 signalling axis (14).

As mentioned earlier, Keap1 acts as a negative regulator of Nrf2 by inhibiting its activity. Nrf2 resides within the cytoplasm, where it associates with Keap1. The interaction between Keap1 and Nrf2 is crucial for preserving cellular equilibrium in OS conditions. Sestrin2, which becomes upregulated in response to OS, enhances the expression of sulfiredoxin by activating the Nrf2 transcription factor. Additionally, Sestrin2 facilitates the activation of Nrf2 through the p62-mediated autophagic degradation of Keap1. Consequently, Sestrin2 mitigates the accumulation of ROS and enhances antioxidant defenses by modulating the Keap1/Nrf2 pathway (3). The binding of p62 to Keap1 sequesters Keap1 within autophagosomes, thereby interfering with the ubiquitin-mediated degradation of Nrf2 and leading to the activation of the Nrf2 signaling pathway. As a substrate regulated by p62, Keap1 undergoes degradation via autophagy. Consequently, p62 plays a critical role in modulating the half-life of Keap1. Elevated levels of p62 markedly decrease the half-life of Keap1, whereas diminished p62 expression results in its prolongation (15). Furthermore, Sestrin2 associates with p62, Rbx1, and Keap1 to assemble a protein complex that facilitates the autophagic breakdown of Keap1 (15). Additional research indicates that p62 engages with the Nrf2-Keap1 interaction domain; therefore, alterations in p62 levels during autophagy—either excess or deficiency—can influence the competition for binding between Nrf2 and Keap1. This modulation affects both the stability of Nrf2 and the transcriptional activation of its downstream target genes (16). Structurally and functionally, p62 possesses an STGE motif that is capable of interacting with the Kelch domain of Keap1 when it is dissociated from the Nrf2-Keap1 complex (15).

This study had several limitations. First, it was conducted exclusively on male Wistar rats, which limits the generalizability of the findings to females or other species, including humans. The duration of the intervention was limited to eight weeks, which may not capture the long-term effects of HIIT and resveratrol supplementation. Additionally, only Keap1 and NRF2 protein levels were assessed, without the evaluation of other related signalling pathways or functional cardiac outcomes. Finally, the study employed a single dose of resveratrol and a single exercise protocol, thereby leaving the effects of different dosages and training intensities unexplored. These limitations should be taken into account when interpreting the results.

Conclusion

The results demonstrated a significant decrease in Keap1 protein levels and a corresponding increase in NRF2

expression in groups undergoing exercise, resveratrol treatment, or both. These findings suggest that both interventions may enhance cardiac antioxidant defense mechanisms by reducing the inhibitory effect of Keap1 on NRF2, thereby promoting NRF2 activation. This aligns with the study's objective to explore how exercise and resveratrol influence the key regulators of OS in the heart, highlighting their potential synergistic role in improving cardiac cellular homeostasis through the modulation of the Keap1-NRF2 pathway.

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Authors' Contribution
Conceptualization: Amir Ahmadiani.
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Competing Interests
The authors declare that there is no conflict of interests to disclose.

Ethical Approval
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