Original Article

The Heart Tissue Molecular Response to Resistance Training in Comparison to Irisin Injection: A Focus on VEGF Gene/Protein Expression and Correlations with Serum Irisin Levels

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Abstract

Background: Angiogenesis is crucial in the recovery and maintenance of heart function. Irisin may mediate the cardiac health-promoting impact of exercise training. The aim of this study was to comparatively assess VEGF gene/protein expression in the heart tissue and correlations with serum irisin levels following resistance training, in comparison to exogenous irisin injection. **Methods:** Twenty-one *NMRI* mice were randomly assigned to the three group ($n = 7$ for each group): control, resistance exercise, and irisin. Exercised mice, for 8 weeks, three sessions per week and four sets of five repeats for each session were considered and mice climbed up a 1-m-height ladder with a slope of 80 degrees with a weight equal to 30% of mouse's body weight fastened to their tails and gradually increased up twofold of body weight. The Irisin group received 100 μg/kg/week irisin for 8 weeks, intraperitoneally. The cardiac expression of the *VEGF* gene, by real-time PCR, the level of VEGF protein, by IHC (immunohistochemistry) and western blot analysis, and serum irisin concentration, by ELISA, were evaluated. **Results:** The expression of the VEGF gene and protein, as well as serum Irisin levels, increased in all experimental mice compared to the control group ($P < 0.05$). Pearson's correlation coefficient data indicated a positive correlation between the analyzed parameters in each group ($P < 0.05$ and $r > 0$). **Conclusions:** There appears to be an interaction between resistance exercise and cardiac angiogenesis factors, mediated by irisin. So, irisin could be considered in cardiovascular health interventions, aiming to target specific molecules or pathways.

Keywords: *Angiogenesis, heart, Irisin, resistance training, VEGF*

Introduction

Cardiovascular diseases, encompassing various conditions that affect the heart and blood vessels, substantially contribute to morbidity and mortality, imposing a significant financial burden.[1] Heart angiogenesis can help in the recovery and maintenance of heart function.[2] Angiogenesis is the intricate process of forming new blood vessels from existing ones and is regulated by pro-angiogenic molecules such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), angiopoietins, interleukin-8 (IL-8), osteopontin (OPN), platelet-derived growth factor (PDGF), transforming growth factor-alpha (TGF-α), hypoxia-inducible factor 1 (HIF-1), epidermal growth factor (EGF), hepatocyte growth factor (HGF), and anti-angiogenic factors, such as endostatin, angiostatin, and thrombospondin-1 (TSP-1).[3,4]

VEGF, belonging to the PDGF supergene family, stands as a crucial pro-angiogenic factor. The VEGF-VEGFR system serves as a significant target for angiogenic-related therapies in cancer, neuronal degeneration, and ischemic diseases.[5] It is reported that the VEGF pathways actively participates in angiogenesis in the myocardial infarction. VEGF influences the migration, proliferation, and decomposition of the extracellular matrix of endothelial cells in various physiological conditions like exercise, embryonic growth, reproductive cycles, wound healing, and pathological status such as tumor growth, ischemia, and pathological hypertrophy of the heart.[5-7]

Regular exercise training has demonstrated the potential to reduce cardiovascular-related challenges.[8] Exercise triggers the production and release of cytokines and other peptides known as myokines, such as interleukin-6 (IL-6), CXCL-1, fibroblast

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growth factor 21 (FGF21), and irisin, which play a pivotal role in generating exercise's effects through their paracrine and endocrine actions.[9-11]

Irisin originates from the proteolytic cleavage of fibronectin type III domain-containing protein 5 (FNDC5), which is primarily expressed in skeletal muscles. Research indicates notable alterations in irisin concentrations within the blood circulation of patients affected by certain cardiovascular diseases compared to their healthy counterparts, suggesting a newfound role of irisin in cardiac health.[11,12] Studies also have demonstrated a substantial surge in irisin levels within the cardiac muscle of both young and elderly rats following exercise.[13] Additionally, regular exercise or irisin supplementation has shown promising improvements in post-myocardial infarction recovery.[14]

Due to its capacity to enhance muscular strength, speed, hypertrophy, balance, and coordination, resistance exercise has garnered increased attention in the last twenty years. Recognized for its numerous health advantages, national health bodies such as the American Heart Association and the American College of Sports Medicine advocate its practice for individuals across various demographics, including the elderly and those grappling with cardiovascular or neuromuscular conditions.[15,16]

Research findings indicate that resistance exercise induces a more pronounced elevation in blood plasma irisin levels compared to endurance-based exercises. The execution of resistance exercises has demonstrated a significant upsurge in the expression of the FNDC5 gene and irisin protein within the hearts.[17] It is shown that irisin has an impact on the expression of VEGF in different tissue such as skin[18] and skeletal muscles,[13] and potentially contributing to the prevention, treatment, and reduction of cardiovascular diseases.[11,12,14] On the other hand, several signaling pathways can influence the production of VEGF such as MAPK (mitogen-activated protein kinase) and muscle hypoxia-related HIF-1α expression. It also has been shown that MAPK activation and HIF-1α expression can be affected by exercise.[19,20]

Hence, this study aimed to comparatively assess VEGF gene/protein expression in the heart tissue and correlations with serum Irisin levels following resistance training, in comparison to exogenous Irisin injection.

Methods

Animal models and tissue preparation

The current study is experimental research involving 21 mature mice (*NMRI*, 5 weeks old, and 18 ± 2 g). These mice were housed in standard conditions within an animal facility, maintaining a temperature of 21°C, a 12:12-hour light/dark cycle, 50 ± 3% humidity, and free access to standard food and water. After a 2-week adaptation period, the mice were randomly divided into three groups: control, resistance exercise, and irisin. The control group received no intervention, while the exercised group underwent a resistance exercise protocol comprising three sessions per week, with four sets of five repetitions in each session. The protocol began with a weight equivalent to 30% of each mouse's body weight attached to their tails, gradually increasing up to twofold of their body weight over an 8-week period. The mice ascended a 1-m-height ladder with an 80-degree slope. Mice in the irisin group received 100 μg/kg/week irisin for 8 weeks, intraperitoneally. Seventy-two hours after the last training session, under anesthesia, blood samples were obtained through cardiac puncture and collected in EDTA tubes. For immunohistochemistry (IHC) analysis, tissue samples were fixed in 10% neutral buffered formalin, while for western blot and real-time PCR analysis, samples were rapidly transferred into liquid nitrogen.[21,22]

ELISA analysis

Blood samples, collected in EDTA tubes, underwent centrifugation at 4000 rpm for 15 min to isolate the serum. Subsequently, the assessment of serum irisin levels was conducted using commercial enzyme-linked immunosorbent assay (ELISA) kits (Aviscera Biosciences, Santa Clara, CA).[23]

Real-time PCR for VEGF gene expression

The quantification of VEGF expression levels was conducted through real-time PCR. Total RNA from each sample was isolated using the Total RNA Prep Kit (BIOFACT, Korea) following the manufacturer's instructions. The purity of the isolated RNA was assessed using the nanodrop 2000 (Thermo Scientific- USA), and DNase I (Sinaclon) was applied to prevent potential genomic DNA contamination. Subsequently, first-strand cDNA synthesis was carried out using The BioFact™ 5× RT Pre-Mix kit (BIOFACT, Korea). The qRT-PCR primers were designed and synthesized using AlleleID 7.6 software (Premier Biosoft) and Metabion (Germany). The primers used were as follows: VEGF forward and reverse primer (CTCAATGTGTCTCTTTGCGCT and GGGGGCTCAGAATCACATCAT, respectively), GAPDH forward and reverse primer (CAGAACATCATCCCAGCCTCC and TTGGCAGGTTTCTCAAGACGG, respectively). Finally, Real-time PCR was performed using the BioFACT™ 2× Real-Time PCR Master Mix kit (BIOFACT, South Korea) and StepOne Plus™ Real-time PCR detection System (Applied Biosystems). GAPDH served as the internal reference.[23]

Immunohistochemistry (IHC) staining

IHC was utilized to assess the VEGF protein levels. The cardiac samples were fixed in 10% natural buffered formalin. Subsequently, a series of alcohol solutions with ascending concentrations up to 100% were employed for dehydration. The samples were embedded in liquid paraffin and sectioned into 4-μm sections. After deparaffinization

with xylene and rehydration, the slides were immersed in 1X TBS (T5912-Sigma), followed by microwave treatment at high power for antigen retrieval. To permeabilize membranes, 3% Triton (Sigma-T8787) was introduced. A 10% goat serum solution (Sigma-G9023) was applied to the samples for 45 min to block the secondary antibody reaction with endogenous mouse IgG. The primary antibody (dilution: 1:100, Santa Cruz, sc-53462) was added, and the slides were incubated for 24 hours at 2-8°C. Subsequently, the secondary antibody (dilution: 1:150, Santa Cruz, sc-2010) was applied, and the slides were incubated for 1 hour and 30 min at 37°C in the dark. The samples were transferred from the incubator to a dark room, and DAPI (D9542- Sigma) was employed for nuclei staining. Fluorescence imaging was conducted using a microscope (Olympus).[24]

Western blot analysis

VEGF protein expression in mouse heart samples was evaluated using the western blot technique, with tissue lysis carried out utilizing RIPA lysis buffer. The quantification of protein levels was conducted using the bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, USA). Following electrophoresis for protein separation on the SDS-PAGE gel, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane. Blocking of the membrane was achieved using 5% skim milk at room temperature, followed by overnight incubation with the Santa Cruz Biotechnology VEGF antibody (orb11554) at 4°C. A secondary antibody (BA1054-2) was then employed to identify the specific antibody complex. To normalize the results, the intensity of the GAPDH band served as an internal control (Anti-GAPDH antibody, GTX100118, Santa Cruz, USA, was used as the primary antibody, followed by a secondary antibody, BA1054-2). Finally, the bands were detected using an ECL western blotting detection system (GE Amersham, UK), and Image J software (NIH, MD, USA) was utilized for densitometry analysis of the protein bands.[24]

Statistical analysis

In this study, statistical analyses were performed using SPSS version 29. The result of all groups was evaluated with the one-way ANOVA test and bivariate associations between serum irisin concentration and VEGF gene/protein expression in the hearts were evaluated by Pearson's correlation coefficient. All data were presented as the mean ± standard deviation (SD). P <0.05 was considered as statistically significant.

Results

The results of all tests are summarized in Table 1.

Serum irisin concentration

Figure 1 depicts the quantitative analysis of serum irisin concentrations using ELISA. Irisin concentration in blood serum showed an increase in exercised mice compared with the control group, which was statistically significant ($P < 0.05$). It was also observed that the level of irisin concentration was significantly elevated in exogenous irisin injection groups compared with the control group ($P < 0.05$). The maximum irisin concentration was seen in the resistance training group but no significant difference was observed between the resistance training group and the irisin injection groups ($P > 0.05$).

VEGF gene and protein expression

The expression of the VEGF gene was assessed using the real-time PCR method. The results indicated that, in comparison to the nonexercised groups, the resistance training group exhibited upregulation of the VEGF gene,

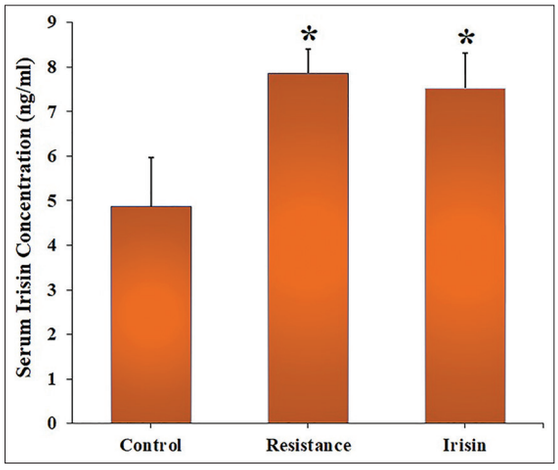

Figure 1: Serum irisin concentration. Irisin concentration increased in resistance training and irisin groups compared to the control group. (*$P < 0.05$) but no significant difference was observed between the exercised mice and irisin-receiving mice ($P > 0.05$). All data were presented as mean ± SD and the acceptable significant difference was at $P < 0.05$

Table 1: Correlation results before and after exercise (n=7 for each group)

Groups	Irisin Concentration		VEGF Gene		VEGF Protein (IHC)		VEGF Protein (Western Blot)	
	Mean±SD	Percentage Change*	Mean±SD	Percentage Change*	Mean±SD	Percentage Change*	Mean±SD	Percentage Change*
Control	4.86±1.1	0	0.0052±0.001	0	13.29±2.8	0	0.489±0.015	0
Resistance Exercise	7.86±0.54	61.72	0.0277±0.014	432.69	47.48±2.7	257.26	0.750±0.034	53.37
Irisin	7.53±0.82	54.93	0.0211±0.008	305.76	41.66±1.8	213.46	0.702±0.035	43.55

*Percentage change compared to the control group

as illustrated in Figure 2. However, this upregulation was statistically significant only when compared to the control group ($P < 0.05$), and no significant difference was observed between the exercised mice and the exogenous irisin injection group ($P > 0.05$). The serum irisin level was significantly increased in the irisin group compared with the control group ($P < 0.05$).

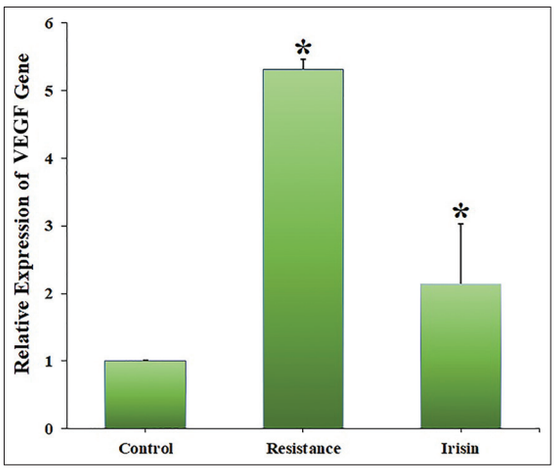

Figure 2: Cardiac VEGF gene expression. In exercise group and exogenous irisin injections group, the VEGF gene expression was significantly upregulated when compared to the control group (*$P < 0.05$). Exercised mice had higher expression of the VEGF gene in comparison with the irisin group but this was not statistically significant. All data were presented as mean ± SD and the acceptable significant difference was at $P < 0.05$

IHC staining for VEGF protein detection showed that VEGF protein was significantly increased in the exercised group and irisin group compared with the control group ($P < 0.05$ in both cases, Figure 3) and the maximum protein level was observed in the trained mice. IHC findings also showed that the expression of VEGF protein was significantly higher in the resistance training group compared with the irisin group ($P < 0.05$).

The western blot analysis [Figure 4] indicated that the VEGF protein expression was significantly higher in all experimental groups than in the control group ($P < 0.05$ in all cases), which confirmed the results of the IHC findings. However, no significant difference was observed between the exercised mice and the exogenous irisin injection group ($P > 0.05$).

VEGF gene/protein expressions and serum irisin levels correlations

Table 2 shows the correlation results in the treated and control groups. A positive correlation was observed between cardiac VEGF gene expression (real-time PCR) and irisin level (ELISA), in the resistance training (r = 0.788, $P < 0.05$) and irisin (r = 0.870, $P < 0.05$) groups. In addition, serum irisin concentration in exercised mice and irisin-receiving mice was found to show a positive correlation with VEGF protein concentration in both IHC (r = 0.836, $P < 0.05$ and r = 0.894, $P < 0.01$,

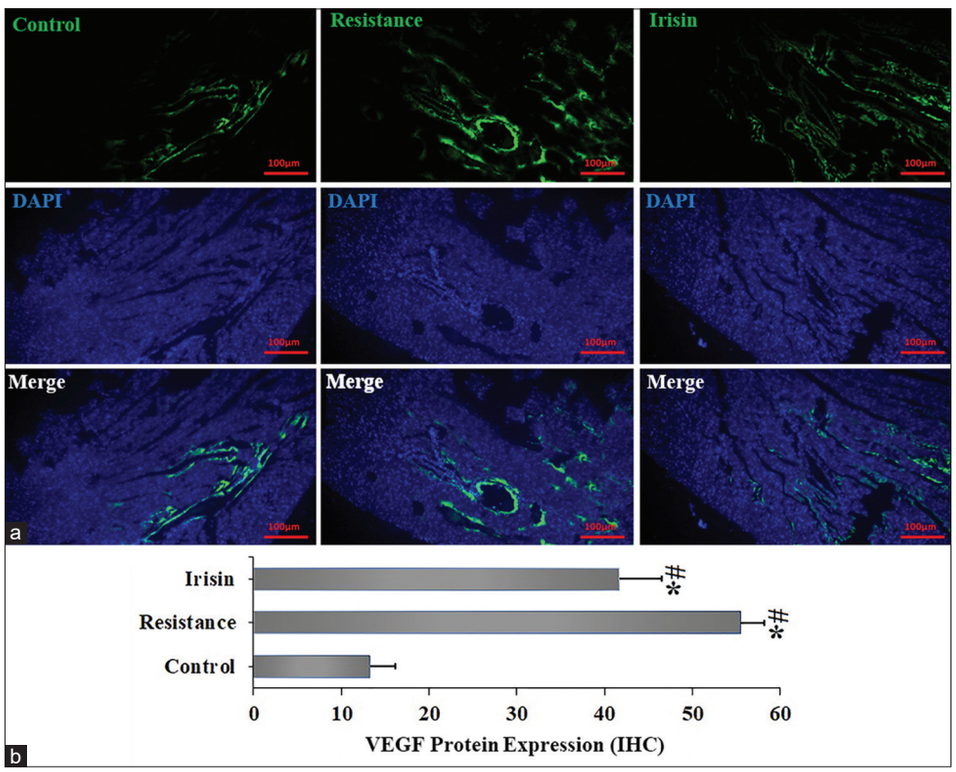

Figure 3: Expression of VEGF protein in heart. (a) IHC images displaying cardiac VEGF protein expression. (b) Quantitative analysis represented that in all experimental groups VEGF protein increased compared to the control group (*$P < 0.05$). The resistance training group had higher statistically significant level of VEGF protein in comparison to irisin group (#$P < 0.05$). All data were presented as mean ± SD and the acceptable significant difference was at $P < 0.05$

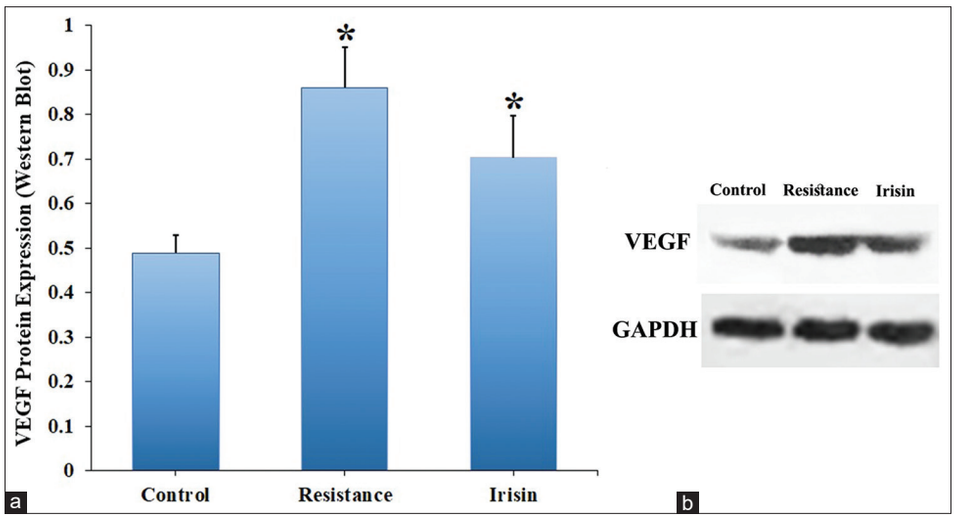

Figure 4: Cardiac expression of VEGF protein assessed through western blot analysis. (a) Quantitative analysis of VEGF protein levels. In comparison with the control group, this protein was significantly upregulated in the resistance training and irisin groups (*$P < 0.05$). No significant differences were observed among treatment groups ($P > 0.05$). (b) Identification of VEGF protein bands using western blot analysis. All data are presented as mean ± SD, and the accepted level of significance was set at $P < 0.05$

Table 2: Correlation results before and after exercise (n=7 for each group)

	Serum Irisin Concentration (ELISA)					
	Control		Resistance Exercise		Irisin Injection	
	R	*P*	*r*	*P*	*r*	*P*
VEGF gene expression (real-time PCR)	0.851	<0.05 (0.015)	0.788	<0.05 (0.035)	0.870	<0.05 (0.011)
VEGF protein expression (IHC)	0.766	<0.05 (0.045)	0.836	<0.05 (0.019)	0.894	<0.01 (0.007)
VEGF protein expression (western blot)	0.817	<0.05 (0.025)	0.917	<0.01 (0.004)	0.871	<0.05 (0.011)

respectively) and western blot tests (r = 0.917, $P < 0.05$ and r = 0.871, $P < 0.01$, respectively). In the control groups, similar significant correlations were found between these parameters.

Discussion

Cardiovascular diseases represent a significant global health concern. A potential approach to enhances heart health is exercise and irisin has been suggested as one of the important exercise mediators for beneficial effects of it.[1,8,11] Angiogenesis, the development of new blood vessels, is recognized as a crucial factor essential for cardiac health, and the VEGF-VEGFR system serves as a significant target for angiogenic-related therapies in various diseases, including ischemia.[4,5]

In this study, resistance exercise significantly increased the level of cardiac VEGF gene and protein expression in comparison with the control group ($P < 0.05$). VEGF is a key regulator of angiogenesis.[4] This underscores the intricate relationship between resistance exercise and molecular adaptations within the heart. A study on the influence of isolated resistance exercise on cardiac remodeling, myocardial oxidative stress, and metabolism in infarcted rats also reported similar findings, indicating that resistance exercise may have beneficial effects on cardiac alterations in infarcted rats.[25] In the present study, elevated expression of VEGF in the cardiac tissue suggests a potential enhancement of vascularization in response to resistance exercise. Xi *et al*.[26] reported that resistance exercise significantly increased the regulation of skeletal muscle Follistatin-like 1 (FSTL1), consequently improving cardiac angiogenesis in subjects with myocardial infarctions. In a study aimed at investigating the effects of different types of exercise (aerobic vs. resistance) on vascular function and VEGF in older women, the serum concentration of VEGF showed an increase following the resistance exercise regimen.[27] However, the mechanism of VEGF gene and protein increase has not been clear in any of the previous studies.

The effects of exercise can be mediated by myokines, types of cytokines and peptides produced by muscle fibers during physical activity, which physiologically connect the organs.[28] The present data demonstrated that resistance exercise increases the level of irisin in the blood of mice. At the molecular level, resistance exercise triggers a cascade of cellular events that lead to an increase in irisin levels in the blood. resistance exercise stimulates skeletal muscle fibers to release myokines, including irisin. Irisin is a cleaved and secreted fragment of the fibronectin type III domain-containing 5 (FNDC5) protein. Muscles release signaling molecules in response to exercise, leading to

an increase in AMP levels and activating AMP-activated protein kinase (AMPK). AMPK stimulates the expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), a transcriptional coactivator that regulates various aspects of energy metabolism. PGC-1α induces the expression of the FNDC5 gene. The FNDC5 protein is cleaved into its active form, irisin. After cleavage, irisin is released into the blood. It is demonstrated that exercise induces a dramatic transient increase in PGC-1alpha transcription and mRNA content in human skeletal muscle. It is reported that high-frequency electrical muscle stimulation, mimicking resistance training, acutely increased the phosphorylation of PKB and the phosphorylation of TSC2, mTOR, and GSK-3β at PKB-sensitive sites, suggesting a specific activation of the AMPK-PGC-1α signaling pathway by resistance-like stimulation.[29,30] Figure 5 provides a summary.

As illustrated in Figure 1, the current study demonstrated that exogenous irisin injection significantly elevated serum irisin levels compared to the control group ($P < 0.05$). Additionally, there was no significant difference between exercised mice and the irisin group ($P > 0.05$). These observations align with findings from prior studies.[17] This suggests that mice subjected to irisin injection may experience similar advantages or complications associated with heightened serum irisin levels, mirroring those undergoing prolonged resistance exercise. In the present study, similar to resistance exercise, irisin injection significantly increased cardiac VEGF gene/protein compared to the control group ($P < 0.05$).

The current study revealed a correlation between serum irisin levels and the expression of both the VEGF gene

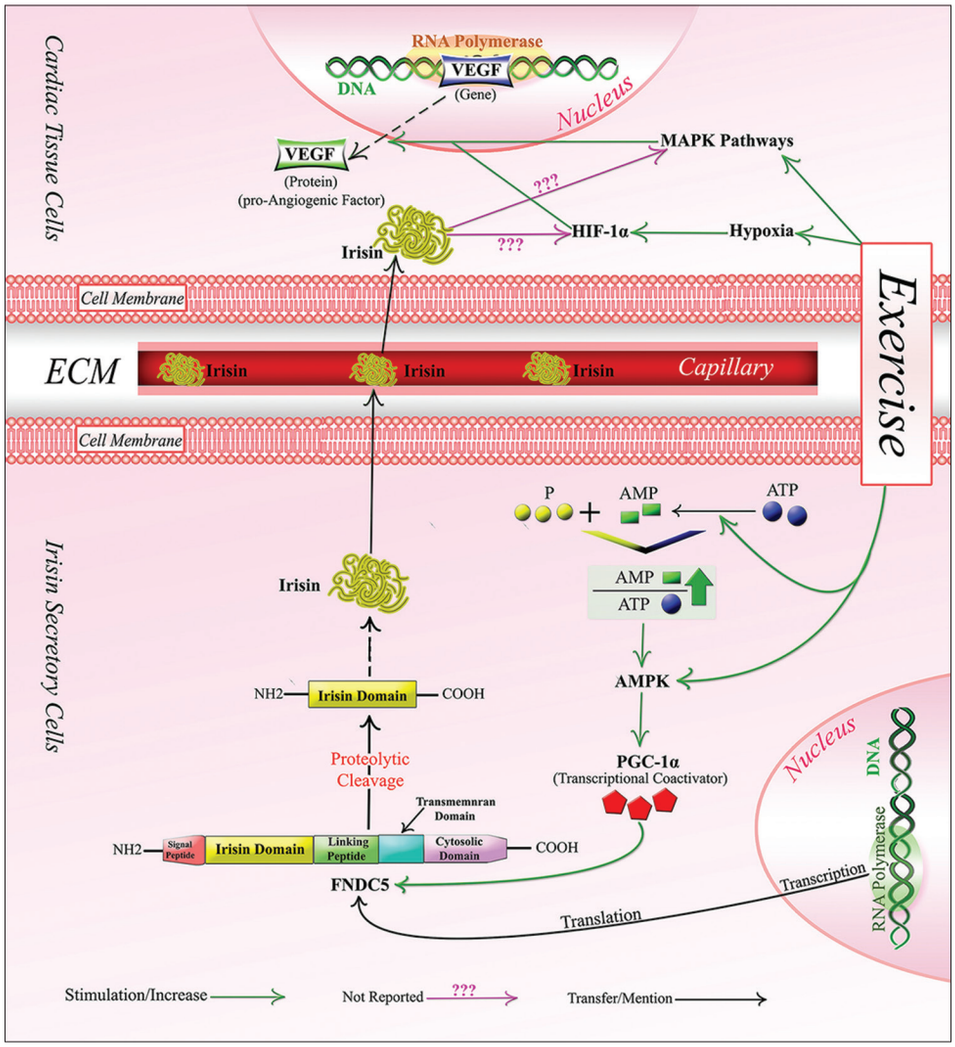

Figure 5: Potential signaling pathways in Irisin-VEGF interaction. Exercise induces an elevation in AMP levels, activating AMPK, which subsequently stimulates the expression of PGC-1α, leading to the induction of FNDC5. The FNDC5 protein is then cleaved into its active form, irisin. The molecular mechanism underlying irisin's effect on VEGF remains unclear. VEGF expression is up-regulated by the MAPK pathway and HIF-1α-related signaling pathways during hypoxia. Exercise can initiate these signaling pathways. It is hypothesized that the relationship between irisin and the VEGF gene/protein may be associated with the signaling pathways linked to MAPK and/or HIF-1α. VEGF, Vascular endothelial growth factor; AMP, Adenosine monophosphate; AMPK, Adenosine monophosphate-activated protein kinase; FNDC5, Fibronectin Type III Domain Containing 5; MAPK, Mitogen-activated protein kinase; HIF-1α, Hypoxia-Inducible Factor-1

and protein in the cardiac tissue in both non-exercised and exercised mice. Previous studies have implicated both irisin and VEGF in exercise-induced cardiac remodeling.[11,31] However, the specific correlation between irisin and VEGF in the context of resistance exercise in *NMRI* mice remains a novel contribution. We suggested that the upregulation of VEGF, a key factor in cardiovascular health, coupled with increased Irisin levels, implies a potential synergistic effect in promoting vascularization and cardiac adaptation and/or a potential mechanism through which irisin may mediate vascular adaptation and cardiac health in response to resistance exercise.

As shown in Figure 5, VEGF production can be influenced by some signaling pathways such as MAPK (mitogen-activated protein kinase). The MAPK pathway is a critical intracellular signaling cascade involved in the regulation of various cellular processes, such as cell proliferation, differentiation, and survival. A study monitored MAPK signaling and found that VEGF secreted from osteocytes activated VEGFR2-MAPK-ERK-signaling pathways in endothelial cells, suggesting the involvement of the MAPK pathway in osteocyte-mediated angiogenesis. It is also reported that the main MAPK pathways, including ERK, JNK, and p38, were differentially activated by cerebral ischemia, with potential implications for neovascularization.[32-34] VEGF expression is also up-regulated during hypoxia by HIF-1α-related signaling pathways in in familial breast cancers,[35] hepatocellular carcinoma,[36] and primary oral melanoma.[37]

Exercise also can induce MAPK activation and muscle hypoxia-related HIF-1α expression. A systematic review evaluated the time course of MAPK phosphorylation in response to resistance exercise, highlighting the impact of resistance exercise on the signaling activities of MAPKs, such as ERK1/2, p90RSK, JNK, and p38-MAPK.[38,39] A study on people with chronic obstructive pulmonary disease (COPD) living at high altitudes suggested the effects of an 8-week program of physical exercise of resistance and muscular strength on HIF-1α.[40] So, it can be suggested that the relationship between irisin and VEGF gene and protein may be related to signaling pathways related to MAPK and/or HIF-1α. However, more studies are needed in the future.

Conclusions

In conclusion, this study demonstrated the dynamic interplay between resistance exercise and cardiac angiogenesis, underscoring the intricate molecular signaling pathways influenced by resistance exercise. Specifically, the potential role of irisin in modulating VEGF-related pathways was highlighted. These findings can be considered in cardiovascular health-related interventions, aiming to address specific molecules or pathways for the development of more effective and targeted treatments.

Acknowledgments

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Ethics statement

The current study received approval from the Research Ethics Committees of the University of Isfahan (Isfahan, Iran, IR.UI.REC.1401.020). All procedures involving animals adhered to the ethical principles and guidelines established for the care and use of laboratory animals.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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References

1. Vaduganathan M, Mensah GA, Turco JV, Fuster V, Roth GA. The Global Burden of Cardiovascular Diseases and Risk: A Compass for Future Health. Washington DC: American College of Cardiology Foundation; 2022. p. 2361-71.
2. Liao Y-Y, Chen Z-Y, Wang Y-X, Lin Y, Yang F, Zhou Q-L. New progress in angiogenesis therapy of cardiovascular disease by ultrasound targeted microbubble destruction. Biomed Res Int 2014;2014:872984.
3. Carmeliet P. Angiogenesis in health and disease. Nat Med 2003;9:653-60.
4. Buschmann I, Schaper W. Arteriogenesis versus angiogenesis: Two mechanisms of vessel growth. News Physiol Sci 1999;14:121-5.
5. Shibuya M. Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: A crucial target for anti-and pro-angiogenic therapies. Genes Cancer 2011;2:1097-105.
6. Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, De Bruijn EA. Vascular endothelial growth factor and angiogenesis. Pharmacol Rev 2004;56:549-80.
7. Farhadi H, Siahkouhian M, Bolboli L, Karimi P. The effect of aerobic training and intermitent hypoxia on expression angiogenic factors in cardiac male wistar rats. University of Mohaghegh Ardabili; 2016.
8. Chen H, Chen C, Spanos M, Li G, Lu R, Bei Y, *et al*. Exercise training maintains cardiovascular health: Signaling pathways involved and potential therapeutics. Signal Transduct Target Ther 2022;7:306.
9. Pedersen L, Pilegaard H, Hansen J, Brandt C, Adser H, Hidalgo J, *et al*. Exercise-induced liver chemokine CXCL-1 expression is linked to muscle-derived interleukin-6 expression. J Physiol 2011;589:1409-20.
10. Ma Y, Kuang Y, Bo W, Liang Q, Zhu W, Cai M, *et al*. Exercise training alleviates cardiac fibrosis through increasing fibroblast growth factor 21 and regulating TGF-β1-Smad2/3-MMP2/9 signaling in mice with myocardial infarction. Int J Mol Sci 2021;22:12341.
11. Zhou X, Xu M, Bryant JL, Ma J, Xu X. Exercise-induced myokine FNDC5/irisin functions in cardiovascular protection and intracerebral retrieval of synaptic plasticity. Cell Biosci 2019;9:32.

12. Ho M-Y, Wang C-Y. Role of irisin in myocardial infarction, heart failure, and cardiac hypertrophy. Cells 2021;10:2103.
13. Aydin S, Kuloglu T, Aydin S, Eren MN, Celik A, Yilmaz M, *et al*. Cardiac, skeletal muscle and serum irisin responses to with or without water exercise in young and old male rats: Cardiac muscle produces more irisin than skeletal muscle. Peptides 2014;52:68-73.
14. Liao Q, Qu S, Tang L-X, Li L-P, He D-F, Zeng C-Y, *et al*. Irisin exerts a therapeutic effect against myocardial infarction via promoting angiogenesis. Acta Pharmacol Sin 2019;40:1314-21.
15. Pollock ML, Gaesser GA, Butcher J, Després JP, Dishman RK, Franklin BA, *et al*. ACSM position stand: The recommended quantity and quality of exercise for developing and maintaining cardiorespiratory and muscular fitness, and flexibility in healthy adults. Med Sci Sports Exerc 1998;30:975-91.
16. Kraemer WJ, Ratamess NA, French DN. Resistance training for health and performance. Curr Sports Med Rep 2002;1:165-71.
17. Dehghan F, Zamani S, Barreiro C, Jami MS. Irisin injection mimics exercise effects on the brain proteome. Eur J Neurosci 2021;54:7422-41.
18. Wang Y, Zhang X, Zhou M, Wu Y, Ma Y, Liu J, *et al*. Irisin pre-treatment promotes multi-territory perforator flap survival in rats: An experimental study. Injury 2020;51:2442-8.
19. Berra E, Pagès G, Pouysségur J. MAP kinases and hypoxia in the control of VEGF expression. Cancer Metastasis Rev 2000;19:139-45.
20. Ramakrishnan S, Anand V, Roy S. Vascular endothelial growth factor signaling in hypoxia and inflammation. J Neuroimmune Pharmacol 2014;9:142-60.
21. Lee S, Farrar RP. Resistance training induces muscle-specific changes in muscle mass and function in rat. J Exerc Physiolgyonline 2003;6:80-7.
22. Colaianni G, Cuscito C, Mongelli T, Pignataro P, Buccoliero C, Liu P, *et al*. The myokine irisin increases cortical bone mass. Proc Natl Acad Sci U S A 2015;112:12157-62.
23. Shahabi S, Esfarjani F, Reisi J, Momenzadeh S, Jami M-S, Zamani S. The effects of 8-week resistance and endurance trainings on bone strength compared to irisin injection protocol in mice. Adv Biomed Res 2021;10:40.
24. Li Q, Guo H, Chou DW, Berndt A, Sundberg JP, Uitto J. Mouse models for pseudoxanthoma elasticum: Genetic and dietary modulation of the ectopic mineralization phenotypes. PLoS One 2014;9:e89268.
25. Rodrigues EA, Lima ARR, Gomes MJ, Souza LM, Pontes THD, Pagan LU, *et al*. Influence of isolated resistance exercise on cardiac remodeling, myocardial oxidative stress, and metabolism in infarcted rats. Antioxidants 2023;12:896.
26. Xi Y, Hao M, Liang Q, Li Y, Gong D-W, Tian Z. Dynamic resistance exercise increases skeletal muscle-derived FSTL1 inducing cardiac angiogenesis via DIP2A–Smad2/3 in rats following myocardial infarction. J Sport Health Sci 2021;10:594-603.
27. Kim H-B, Seo M-W, Jung HC. Effects of aerobic vs. resistance exercise on vascular function and vascular endothelial growth factor in older women. Healthcare 2023;11:2479.
28. Sabaratnam R, Wojtaszewski JF, Højlund K. Factors mediating exercise-induced organ crosstalk. Acta Physiol 2022;234:e13766.
29. Pilegaard H, Saltin B, Neufer PD. Exercise induces transient transcriptional activation of the PGC-1α gene in human skeletal muscle. J Physiol 2003;546:851-8.
30. Atherton PJ, Babraj JA, Smith K, Singh J, Rennie MJ, Wackerhage H. Selective activation of AMPK-PGC-1α or PKB-TSC2-mTOR signaling can explain specific adaptive responses to endurance or resistance training-like electrical muscle stimulation. FASEB J 2005;19:786-8.
31. Rinaldi B, Donniacuo M, Sodano L, Gritti G, Signoriello S, Parretta E, *et al*. Effects of sildenafil on the gastrocnemius and cardiac muscles of rats in a model of prolonged moderate exercise training. PLoS One 2013;8:e69954.
32. Lennmyr F. Signal transduction in focal cerebral ischemia: Experimental studies on VEGF, MAPK and Src family kinases. Acta Universitatis Upsaliensis; 2002.
33. Quan J-H, Ismail HAHA, Cha G-H, Jo Y-J, Gao FF, Choi I-W, *et al*. VEGF production is regulated by the AKT/ERK1/2 signaling pathway and controls the proliferation of toxoplasma gondii in ARPE-19 cells. Front Cell Infect Microbiol 2020;10:184.
34. Prasadam I, Zhou Y, Du Z, Chen J, Crawford R, Xiao Y. Osteocyte-induced angiogenesis via VEGF–MAPK-dependent pathways in endothelial cells. Mol Cell Biochem 2014;386:15-25.
35. Saponaro C, Malfettone A, Ranieri G, Danza K, Simone G, Paradiso A, *et al*. VEGF, HIF-1α expression and MVD as an angiogenic network in familial breast cancer. PLoS One 2013;8:e53070.
36. Ren F, Wu K, Yang Y, Yang Y, Wang Y, Li J. Dandelion polysaccharide exerts anti-angiogenesis effect on hepatocellular carcinoma by regulating VEGF/HIF-1α expression. Front Pharmacol 2020;11:460.
37. Simonetti O, Lucarini G, Rubini C, Goteri G, Zizzi A, Staibano S, *et al*. Microvessel density and VEGF, HIF-1α expression in primary oral melanoma: Correlation with prognosis. Oral Dis 2013;19:620-7.
38. Lee CJ, Nicoll JX. Time course evaluation of mitogen-activated protein kinase phosphorylation to resistance exercise: A systematic review. J Strength Cond Res 2023;37:710-25.
39. Ji X, Xu Z, Liu D, Chen Y. Effects of exercise training on neurological recovery, TGF-β1, HIF-1α, and Nogo-NgR signaling pathways after spinal cord injury in rats. Clinics 2023;78:100236.
40. Villamil-Parra W, Cristancho-Mejía É, Ramon Torrella J, Mancera-Soto EM. Effects of a physical exercise program on HIF-1α in people with chronic obstructive pulmonary disease living at high altitude: Study protocol for a clinical trial. Trials 2023;24:698.