Research Article

Correlation between the muscle, blood and heart level of Irisin in exercise-trained rats with Nano selenium supplementation: A rat model of COPD

Zohreh Fathi[1], Javad Raouf Sarshoori[2], Mohammad Reza Masjedi[3], Shadmehr Mirdar[1*]

Abstract

The aim of this study was to considering the correlation between the muscle fibronectin type III domain-containing protein 5 (FNDC5), blood and heart level of Irisin in exercise-trained rats with Nano selenium supplementation after intraperitoneal injection of cigarette smoke extract induced chronic obstructive pulmonary disease (COPD). To this end, 49 male Wistar rats (8 weeks old) were divided into seven groups: control, SeNPs (2.5 mg/kg b.w by oral gavage, 3 days/week, 6 weeks), AIT (49 min/day, 5 days/week for 6 weeks, interval), SeNPs+AIT, CSE (150 µL by IP injection, 1 day/week for 6 weeks), CSE+AIT, and CSE+SeNPs+AIT. The results of the present study showed that CSE injection caused inflammation and damage to lung tissue, especially alveoli, compared to the healthy group. In other words, based on the histological examination of cigarette smoke extract, it was able to cause lung tissue damage similar to COPD, and doing exercise and taking nanoselenium antioxidant supplement could control these lung tissue damage. Pearson's correlation method was used to investigate the relationship between muscle FNDC5, serum and heart Irisin, and the results of this correlation were not significant in different groups ($p>0.05$). It seems that exercising and taking nanoselenium supplements can increase Irisin levels in serum and heart tissue by expanding muscle contraction and increasing muscle FNDC5. However, the relationship of this factor in muscle and heart crosstalk should be investigated more closely.

Key Words: **Irisin, Skeletal muscle, Heart, Aerobic exercise, Nanoparticles, Cigarette smoking, FNDC5**

Introduction

Disorders in the structure and function of skeletal muscles are common in patients with chronic obstructive pulmonary disease (COPD) (Taivassalo & Hepple, 2022). Lack of movement caused by COPD can eventually cause a decrease in load and muscle contraction and eventually atrophy of muscle tissue (Wu et al., 2023). In these patients, muscle strength and endurance decrease, while muscle fatigue increases. Type I muscle fibers also decrease and type II increases. In other words, muscle atrophy occurs with a decrease in the cross-sectional area of the fiber (Mathur et al., 2014). At the tissue and cellular level, oxidative enzyme activity decreases and muscle bioenergetic measurement during exercise shows a decrease in aerobic capacity (Plant et al., 2010). In addition to the skeletal muscle, the heart muscle and the heart tissue itself are also affected by COPD. In addition to these cellular changes, muscle atrophy can lead to a decrease in the secretion of myokines, including irisin (Reza et al., 2017), which is harmful to various tissues, including the heart (Lecker et al., 2012).

Irisin is a myokine that is obtained from the cleavage of fibronectin type III domain-containing 5 (FNDC5). Irisin, which is mostly secreted from muscle tissue, regulates mitochondrial energy, glucose metabolism, fatty acid oxidation, and fat browning. Based on studies, skeletal muscles and hearts produce irisin and affect various cardiovascular functions (Ou-Yang et al., 2021). In the early stage of acute myocardial infarction, increasing irisin levels can reduce endothelial damage by inhibiting inflammation and oxidative stress (Ho & Wang, 2021). In other words, irisin has different effects on mitochondrial dysfunction, oxidative stress, metabolic imbalance, energy consumption and prognosis of heart failure (Piątek et al., 2019). Irisin affects blood pressure and controls blood pressure by modulating vasodilation. In addition, irisin can increase vasoconstriction through the hypothalamus. Due to these dual effects of irisin on cardiovascular physiology, irisin can be a vital therapeutic target in cardiovascular diseases, especially in COPD patients.

1. Department of Exercise Physiology, Faculty of Physical Education and Sport Sciences, University of Mazandaran, Babolsar, Iran. 2. Anatomy Department of Medical Faculty, Baqiyatallah University of Medical Science, Tehran, Iran. 3. Tobacco Control Research Center (TCRC), Iranian Anti-Tobacco Association, Tehran, Iran

*Author for correspondence: shadmehr.mirdar@gmail.com

Z F: 0000-0001-6897-7764; J R S: 0000-0001-8155-1573; MRM: 0000-0002-6871-382X; Sh M: 0000-0003-3075-1529

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Selenium is one of the elements that are useful for the body. Selenium has many antioxidant effects (Jenkins et al., 2020). In cardiac tissue, selenium has been shown to scavenge reactive oxygen species and potentially prevent cardiac hypertrophy. Selenium nanoparticles (SeNP), composed of spherical microparticles containing selenium, may be a more effective way to deliver selenium as an antioxidant to prevent cardiac damage (Al-Mubarak et al., 2021). Therefore, in the present study, SeNP is used along with exercise to control the damage caused by cigarette smoke. In other words, in this study, Irisin changes in muscle, serum and heart in COPD model caused by cigarette smoke extract are evaluated.

Materials and Methods

Animals

Experiments were performed according to NIH Guidelines for animal studies on male Wistar rats weighing about 180–220 g (age: 8 weeks) that were bred in the animal laboratory, at Baqiyatallah University of medical sciences, Tehran, Iran (ethical code: IR.BMSU.REC.1400.117). The animals were maintained under controlled conditions (a 12 h light-dark cycle at 22 °C) with ad libitum access to food (standard chow diet: protein, 23%; fat, 4%; carbohydrate, 42%; calcium, 1%; tryptophan, 0.25%; methionine, 0.33%; lysine, 1.15%; threonine, 0.7%; phosphorus, 0.65%; salt, 0.5%; fiber, 4%; energy (Kcal/kg), 2900) and water. Forty-nine male Wistar rats were randomized into seven groups:

1. Control group: received 150 microliters (µL) of vehicle (normal saline) intraperitoneally.

2. SeNPs group: received 2.5 mg/kg b.w. of this supplement by stomach gavage, 3 days/week for 6 weeks.

3. AIT group: performed aerobic interval training on a rodent treadmill 5 days/week for 6 weeks (in the form of intervals for 7 sets: each set of intervals: 4 min at 80-90% VO2max, separated by 3 min at 65-75% VO2max).

4. SeNPs+AIT group: received SeNPs and performed AIT (as stated in groups 2 and 3).

5. CSE group: received 150 µL CSE by IP injection, 1 day/week for 6 weeks.

6. CSE+AIT group: received CSE and performed AIT (as stated in groups 3 and 5).

7. CSE+SeNPs+AIT group: received SeNPs, CSE, and performed AIT (as stated in groups 2, 3 and 5).

Cigarette smoke extract (CSE)

CSE was prepared based on previously relevant studies (Chen et al., 2015; Li et al., 2018). Briefly, three Winston cigarettes (con-taining tar, 12 mg, nicotine, 0.9 mg) were burned and the smoke was transferred to a container containing 10 ml of PBS using a vacuum pump. The CSE-PBS solution for each set of experiments was freshly prepared and then filtered through a 0.22 mm Millipore filter to remove particles and bacteria. The rats of the healthy control group were given 150 microliters (µL) of vehicle (normal saline) intraperitoneal, while the CSE groups were given 150 microliters (µL) of CSE-PBS solution on days 7, 14, 21, 28, 35, and 42 which were injected intraperitoneal (Chen et al., 2015; Li et al., 2018).

Nano selenium supplementation

In this study, a nano-selenium manufactured by ARMINANO company was utilized (Armina Engineering Co, Tehran, Iran). To prepare the mixture, firstly based on the company description, the aqueous extract of ginger obtained was utilized as a precursor for the synthesis of nano-selenium. Ginger extract (2 ml) was added dropwise into the 20 ml solution of SeO3 (10 mM), with vigorous stirring. The mixture was incubated by placing the solution onto a rotatory orbital shaker operating at 200 rpm, 30 °C for 72 h in dark conditions. The reduction of selenium ions was monitored by sampling an aliquot (3 ml) of the mixture at intervals of 24 h, followed by measurement of absorption maximum. Absorption maximum was determined by measuring the optical density of the content from wavelength 350 to 700 nm using UV–Vis spectrophotometer (Abou Zaid et al., 2017; Hozyen et al., 2020; Prasad & Selvaraj, 2014). SeNPs at a dose (of 2.5 mg/kg b.w) were given to the supplementation group by oral gavage 1 a day for 3 times/week (Ali et al., 2020).

Aerobic interval training

Prior to the onset of the main training protocol and to get familiar with treadmill running, the rats exercised for five min at a speed of 8-10 m/min with a zero slope in five sessions in one week. The aerobic interval training (AIT) protocol consisted of a ten-min warm-up (50-55% of maximal oxygen uptake (VO2max)), seven periods of interval training (each set of intervals: 4 min at 80-90% VO2max, separated by 3 min at 65-75% VO2max) and 5-min cool down (Qin et al., 2020). To adjust the running speed and maintain the relative training intensity, VO2max indirect measurement test was employed for Wistar rats once every two weeks.

ELISA

Forty-eight h after the last training session, rats were anesthetized with an acute injection of xylazine/ ketamine (10 mg/kg/100 mg/kg). The blood sample was collected by cardiac puncture into non-heparinized tubes and then centrifuged at 3000 rpm for 15 min. Sera were meticulously separated and each sample was put in a clean cup tube, labeled, and kept at -20°C until biochemical analysis. Serum and heart irisin were measured using rat-specific ELISA kits according to the manufacturer's inst-

-ructions (Irisin: ZellBio GmbH, Germany). The studies were carried out using an ELISA plate washer (BIOTEK ELx50, USA) and ELISA microplate reader (ELX808; BioTek Instruments).

Gene expression

Using Qiazol (Qiazol lysis reagent, USA), the extraction of the whole RNA was performed from gastrocnemius muscle and lung tissue, within a sterilized RNase-free tube. The RNA purity and concentration were measured utilizing the absorbance at 260 and 280 nm ratio (the A260/280 ratio) through a Nano Drop ND-100 spectrophotometer (Thermo Scientific, USA). The Revert aid cDNA synthesis kit (Fermentas, Germany) was used to transform RNA into cDNA in a quantity of 25 µL, in accordance with the manufacturer's instructions. Polymerase chain reaction (PCR) amplification reaction included 2 µl of the cDNA synthesis reaction, 12.5 µl AccuPrime SuperMix I (Fermentas, Germany), 10.1 µl of distilled water, and 0.2 µl of each forward and reverse primers (100 µmol/L). The NCBI BLAST Instrument and Primer3 software were applied to design and confirm primers.

For real-time PCR, 500 ng of the newly synthesized cDNA was used to assess the relative gene expression. PCR reactions were carried out utilizing SYBR Green Premix 2× (12.5 µL; Takara, Japan) and mixed primers (10 p-molar; 25 µL). The thermal cyclic protocol utilized was 95 °C for 10 s, 40 denaturation cycles at 94 °C for 5 s, and annealing and extension at 60 °C for 34 s. The

Table 1. Primer sequences.

Gene	Primer sequences
Muscle FNDC5	F: 5'- AGGACCTCACTGTTCTGACG -3'
	R: 5'- GCAGTCTTGTCACTCCAGGA -3'
GAPDH	F: 5' ATCAAGAAGGTGGTGAAGCAGG 3'
	R: 5' TGGGAGTTGCTGTTGAAGTCAC 3'

ΔΔCT technique was utilized to quantify the muscle and lung FNDC5 gene relative expressions. A comparison was made between the samples of Ct and that of the internal control (Gapdh). Real-time PCR was conducted using the ABI (Applied Biosystems, USA) detection system. All reactions were completed five times. Using electrophoresis and melting curve analysis, the specificity of the PCR reaction was double-checked. Graph pad prism5 was used for gene analysis. The employed primer sequences are depicted in Table 1.

Hematoxylin and Eosin (H&E) analysis (lung tissue)

The H&E method was used to investigate lung tissue damage caused by CSE. A piece of the lung tissue was fixed in 10% formalin for 24 h. H&E staining was performed using standard procedures. Pulmonary tissue morphology was observed under a DP73 digital microscope after paraffin embedding, sectioning, and H&E staining.

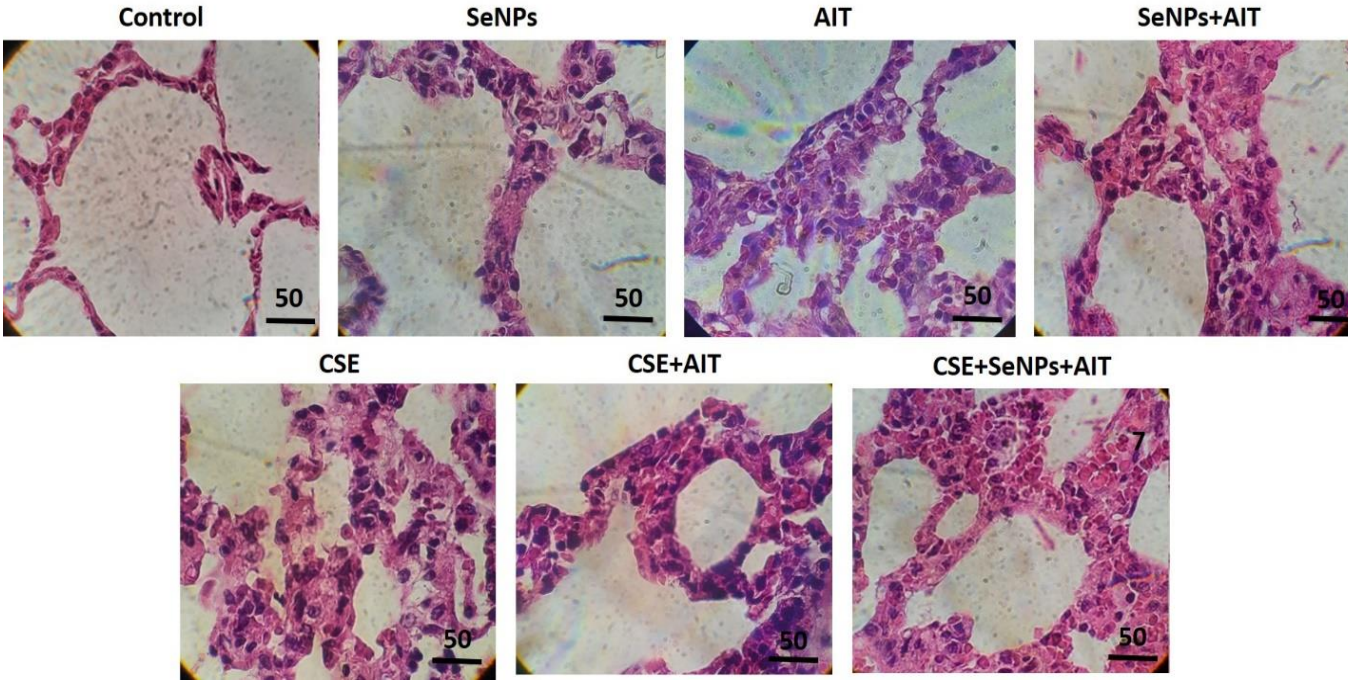

Figure 1. Lung alveoli of healthy and cigarette smoke extract-exposed rat after the exercise and Nano selenium supplementation. Magnification: 40 x-scale bar 50 µm. H&E stain. SeNPs: Nano-selenium, AIT: Aerobic interval training, CSE: Cigarette smoke extract.

Statistical analysis

Data are expressed as mean ± SD. Shapiro-Wilk test was conducted to confirm the normality of the data. The Pearson's correlation was used to analyze the correlations of FNDC with the concentrations of serum Irisin, serum Irisin and heart Irisin separately (Graph Pad Prism version 9.01). The significance level was considered at the α level of 0.05.

Results

Lung histology

Lung tissue (Alveoli) of rats in different healthy and cigarette smoke extract groups are shown in Figure 1. As can be seen, the healthy groups have more tissue cohesion than the CSE groups. CSE injection has caused the tissue destruction of inflammation and the infiltration of lymphocytes into the lung tissue, as well as disturbed the integrity of the lung. While regular exercise and the use of Nano-selenium supplements have neutralized these damages to some extent.

Serum and heart Irisin correlation

Pearson's correlation was used to investigate changes in serum and heart Irisin levels in different research groups (Figure 3). As can be seen, the healthy groups, especially the AIT group, showed a positive correlation between serum and heart Irisin levels, but these changes were not significant (p>0.05). In the treated groups, although serum and heart Irisin levels were increased in the exercise and Nano supplement groups, these changes were not significant (p>0.05).

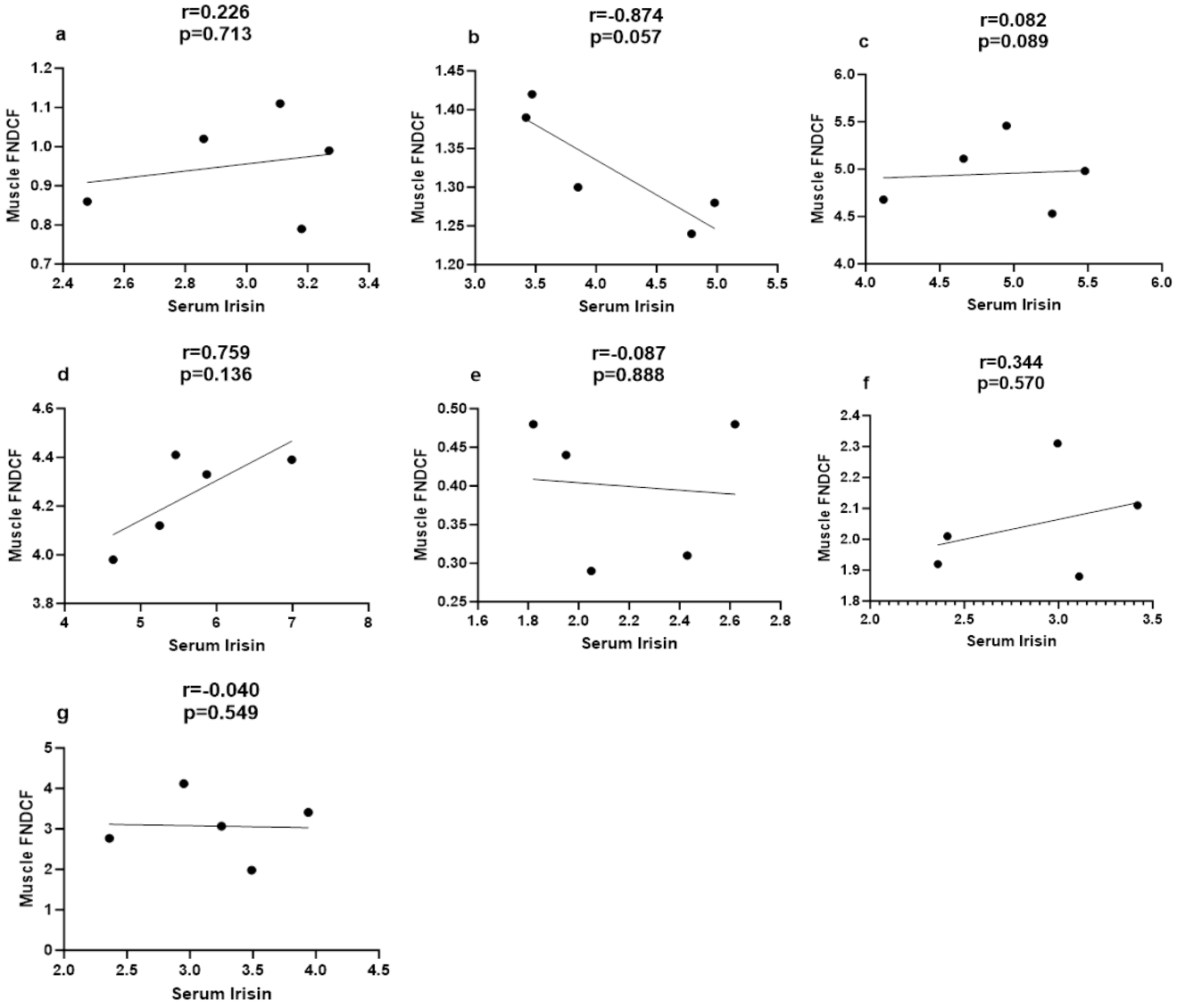

Figure 2. Muscle FNDC5 and serum Irisin correlation at different groups of study (a: Control, b: SeNPs, c: AIT, d: SeNPs+AIT, e: CSE, f: CSE+AIT, g: CSE+SeNPs). Data are expressed as mean ± standard deviation. SeNPs: Nano-selenium, AIT: Aerobic interval training, CSE: Cigarette smoke extract.

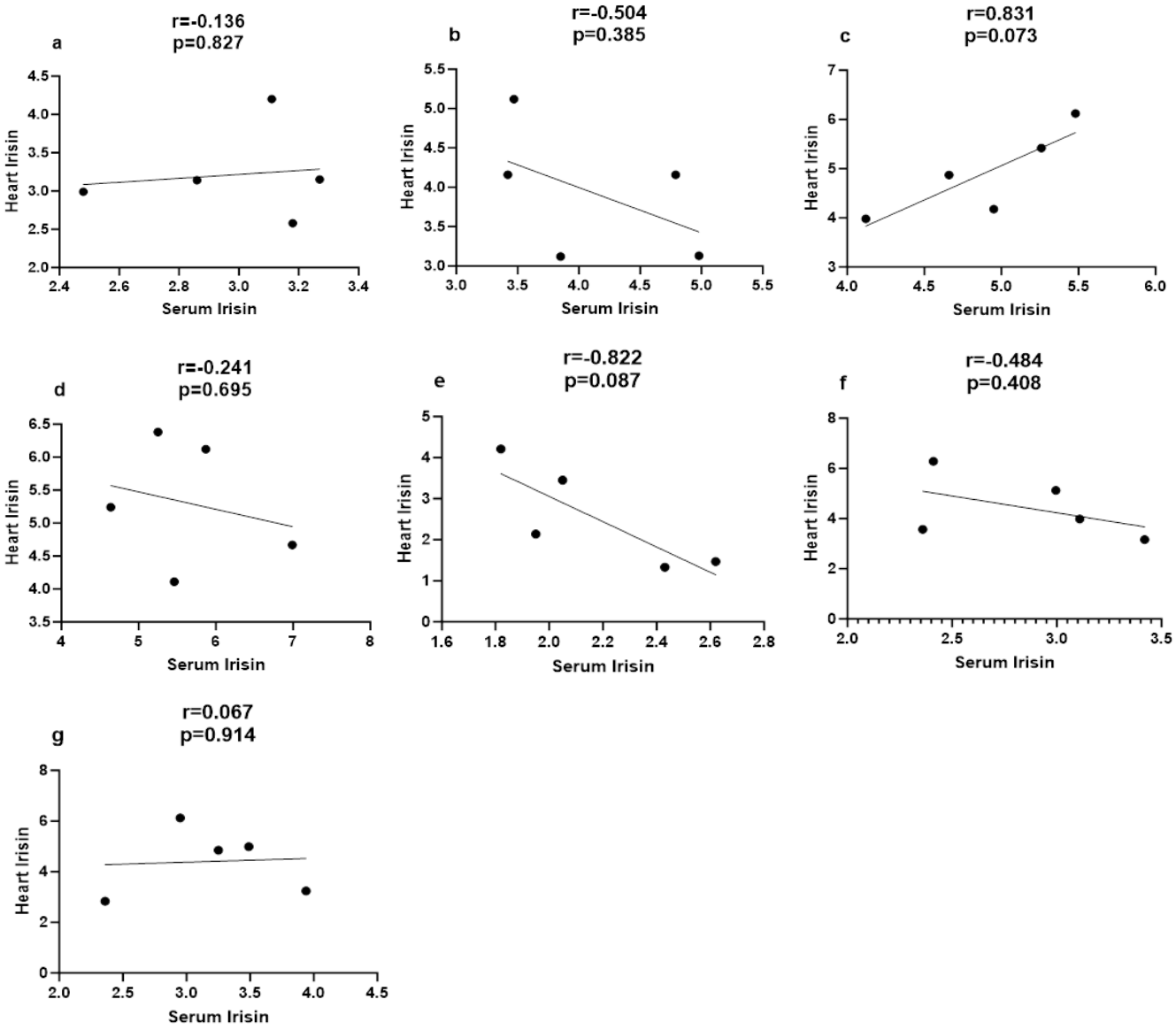

Figure 3. Heart and serum Irisin correlation at different groups of study (a: Control, b: SeNPs, c: AIT, d: SeNPs+AIT, e: CSE, f: CSE+AIT, g: CSE+SeNPs). Data are expressed as mean ± standard deviation. SeNPs: Nano-selenium, AIT: Aerobic interval training, CSE: Cigarette smoke extract.

Serum and heart Irisin correlation

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Discussion

In heart and lung diseases, the serum and tissue levels of irisin usually decrease, and the increase of this factor can be introduced as a therapeutic target. Skeletal muscle is one of the most important serum sources of Irisin, which can have an endocrine effect on other tissues. The purpose of this study was to investigate the effect of exercise and Nano selenium supplementation on skeletal muscle FNDC5, serum and heart Irisin levels in COPD model, and the correlation of this factor was evaluated.

At first, the hematoxylin-eosin method was used to confirm the induction of COPD and lung tissue damage after the injection of cigarette smoke extract. Histological results confirmed the damage of pulmonary alveoli after CSE injection, while exercise training and especially Nano selenium supplementation reduced the damage caused by CSE. Cigarette smoke extract is a mixture of chemicals that have direct and/or indirect toxic effects in the lu-

-ng cells. Cigarette smoke is a potent source of oxidative stress, various DNA-damaging and apoptosis-inducing factors for HFL-1 cells, and it is thought that cigarette smoke can contribute to the development of pulmonary emphysema in the lungs of smokers. Cigarette smoke is the main cause of emphysema. Epidemiological and clinical studies have shown that smokers are significantly more likely to develop emphysema than non-smokers, and the severity of the disease is directly related to the amount of smoking (Nakamura et al., 1995). Among the various toxic effects of cigarette smoke on human tissues, the oxidation of structural and functional molecules is an important damage (Repine et al., 1997). Kasahara et al (Kasahara et al., 2001) showed that cigarette smoke may act by reducing the expression of vascular endothelial growth factor (VEGF) and its receptor type 2, thereby leading to the death of lung septal endothelial cells. Therefore, lung tissue damage seems logical according to the description of these mechanisms. In other words, the toxins in cigarette smoke can be absorbed into the blood stream through the peritoneum and transferred to different tissues, including the lungs. Among the important effects of Nano-selenium supplement is its antioxidant property, which seems to be able to combat the oxidative damage caused by cigarette smoke by increasing its antioxidant capacity along with exercise. In other words, exercise overproduces reactive oxygen species (ROS) and eventually exceeds the body's antioxidant capacity to neutralize them (Kamareh et al., 2023). ROS have damaging effects on cell membranes and contribute to skeletal muscle damage. Selenium (Se), a natural mineral trace element, is an essential component of selenoproteins, which plays an important role in antioxidant defense. The activity of glutathione peroxidase (GPx), a highly efficient antioxidant enzyme, is closely dependent on the presence of Se. These properties of Se may potentially be used to improve athletic performance and exercise recovery (Fernández-Lázaro et al., 2020). However, oxidative and antioxidant changes were not investigated in this study.

Among the hypotheses of the current research was the investigation of muscle, serum and heart changes of Irisin, and the relationship of this factor in three paths was investigated by correlation method. Although the changes of muscle FNDC5 and serum and cardiac Irisin levels increased in the training and Nano supplement groups, but when the correlation was examined in each group separately, the relationship between muscle FNDC5 and serum Irisin, as well as the relationship between serum Irisin and cardiac Irisin in none of them was not confirmed. Contrary to the results of the present study, Arabzadeh et al (2024) confirmed the relationship between serum Irisin and lung FNDC5 in the exercise and Nano supplement groups (Arabzadeh et al., 2024). However, lung tissue was not evaluated in the present study. It seems that the smallness of the statistical population (5 rats in each group) is one of the main reasons for this non-significant difference, and it is suggested that a larger number of samples should be used in future studies to check the correlation.

Conclusion

It seems that although exercise training and Nano selenium supplementation are able to increase the serum and heart levels of Irisin, their relationship to confirm the hypothesis of heart muscle cross talk is limited. However, the researchers suggest further research with a higher statistical population to investigate this relationship in detail.

What is already known on this subject?

Irisin, which is mostly secreted from muscle tissue, regulates mitochondrial energy, glucose metabolism, fatty acid oxidation, and fat browning.

What this study adds?

Exercise training and Nano selenium supplementation are able to increase the serum and heart levels of Irisin.

Organ Cross-Talk Tips:

- In the lung disease, exercise training has this potential increase the crosstalk between muscle and heart that reduce the injuries of lung on the heart tissue.

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Funding

None.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Experiments were performed according to NIH Guidelines for the animal study on Wistar rats weighing about 180–220 g bred at the animal laboratory, at Baqiyatallah University of medical sciences, Tehran, Iran (ethical code: IR.BMSU.REC.1400.117).

Informed consent Animal study.

Author contributions

Conceptualization: Z.F.; Methodology: M.R.M., J.R.S.; Software: Z.F., Sh. M.; Validation: Sh.M.; Formal analysis: Z.F.; Investigation: Sh.M.; Resources: M.R.M.; Data curation: J.R.S.;

Writing - original draft: Z.F.; Writing - review & editing: Sh.M.; Visualization: M.R.M.; Supervision: Sh.M.; Project administration: Z.F.; Funding acquisition: M.R.M.

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