

Original Article



Effect of Aerobic Exercise and Capsaicin Supplementation on Estrogen Receptor 1 Gene Expression in Skeletal Muscle and Serum Calcium Levels in Aged Rats

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Abstract

Introduction: Physical activity and nutritional interventions are effective strategies for mitigating the effects of aging. Estrogen Receptor 1 (*ESR1*) is one of the two main types of estrogen receptor proteins. The present study aimed to investigate the effects of aerobic exercise and capsaicin supplementation on *ESR1* gene expression in skeletal muscle and serum calcium levels in aged rats.

Methods: In this experimental study, 32 Wistar rats aged 20 months were randomly divided into four groups (n=8): aerobic exercise, capsaicin supplementation, aerobic exercise + capsaicin supplementation, and a control group. The aerobic exercise protocol was performed for eight weeks, five sessions per week. In the first week, rats ran on a treadmill for 10 minutes at 40% of their maximum speed, with intensity gradually increased to 30 minutes at 80% of maximum speed by the final week. Capsaicin (5 mg/kg) was administered by gavage for eight weeks, five days per week. Data were analyzed using one-way ANOVA ($P \leq 0.05$).

Results: *ESR1* gene expression significantly increased in the exercise (1.53 ± 0.021), capsaicin (1.40 ± 0.013), and combined intervention groups (2.06 ± 0.037) compared with the control group (0.29 ± 0.087) ($P < 0.05$). The combined intervention showed a greater effect than either treatment alone. Serum calcium levels significantly increased in the capsaicin (2.62 ± 0.28) and combined groups (2.20 ± 0.19) compared with the control group (1.36 ± 0.11) ($P < 0.05$); however, the increase in the exercise group (1.47 ± 0.23) was not statistically significant.

Conclusion: Aerobic exercise combined with capsaicin supplementation may improve muscle function by upregulating *ESR1* gene expression and improving calcium regulation in aged skeletal muscle.

Keywords: Capsaicin, Exercise, Estrogen receptor, Rats

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Introduction

The aging population in Iran, similar to other countries, is increasing due to declining birth rates, improved healthcare, and increased life expectancy. This demographic shift necessitates addressing the specific health and functional challenges faced by the elderly (1). Aging is a complex process characterized by various progressive physiological changes (2). A key aspect of this is skeletal muscle decline, or sarcopenia, characterized by reduced muscle mass and function resulting from fiber atrophy, decreased levels of anabolic hormones such as growth hormone and Insulin-like Growth Factor-1 (IGF-1), and increased non-contractile tissue density (3). Beginning in the fourth decade of life, sarcopenia leads to muscle weakness, a loss of independence, an increased risk of falls and fractures, and reduced quality of life, ultimately contributing to higher morbidity and mortality (3). Aging is generally classified into primary aging, driven by genetic factors, and secondary aging, which is influenced by environmental and lifestyle factors such as nutrition, physical activity, and disease (1).

Several strategies have been proposed to mitigate muscle atrophy, including nutrition, pharmacological interventions, and physical activity. Protein or essential

amino acid intake stimulates muscle protein synthesis, while antioxidant supplementation can counteract oxidative stress, a key contributor to age-related muscle loss (1, 3). Exercise further enhances skeletal muscle metabolism by activating signaling pathways that improve contractility and physiological function (3). Additionally, maintaining adequate levels of calcium and phosphorus is essential for bone health and serves as primary indicators of fracture risk (2).

Estrogen exerts its physiological effects through three receptors: Estrogen receptor α (ER α), ER β , and the G protein-coupled membrane estrogen receptor (4). ER α , encoded by the *ESR1* gene, is a ligand-dependent transcription factor widely expressed in tissues such as skeletal muscle, bone, the heart, and adipose tissue (5). Estrogen and its receptors are crucial for reproductive function and also play important roles in muscle and bone homeostasis, contributing to hypertrophy and the maintenance of bone mass (4). Increased *ESR1* expression in skeletal muscle is linked to improved mitochondrial function and metabolic regulation. Regular exercise has been shown to enhance antioxidant defenses, reduce inflammation, and upregulate *ESR1* expression (6, 7).

Aerobic exercise, in particular, is an effective strategy for preventing age-related functional decline, while weight-bearing exercise helps preserve bone mass in the elderly (8).

Nutritional interventions with antioxidant and anti-inflammatory properties have also been explored. Capsaicin, the main bioactive compound in red pepper, has been reported to stimulate skeletal muscle activity, increase intracellular calcium, promote hypertrophy, enhance endurance, and improve metabolic function (9, 10). Its antioxidant and cardiometabolic benefits further support its potential as a therapeutic agent against age-related muscle decline.

Despite existing evidence on the independent effects of aerobic exercise and capsaicin, their combined impact on *ESR1* gene expression in skeletal muscle remains under-investigated, and previous findings are sometimes contradictory. Therefore, this study aimed to examine the effects of aerobic exercise and capsaicin supplementation on *ESR1* gene expression and serum calcium levels in aged rats.

Materials and Methods

In this experimental study, 32 male Wistar rats (aged 20 months, weighing 200–220 g) were purchased from the Pasteur Institute and transferred to the animal laboratory at Baqiyatallah University of Medical Sciences. Following a one-week acclimatization period, the rats were randomly assigned to one of four groups ($n=8$ per group) using an online randomization software: control, aerobic exercise, capsaicin supplementation, and aerobic exercise plus capsaicin supplementation. During the experimental period, the rats were housed in transparent polycarbonate cages (manufactured by Razi Rad Company; dimensions: $15 \times 15 \times 30$ cm) with five rats per cage. The housing environment was strictly controlled, maintaining a 12:12-hour light–dark cycle, an ambient temperature of $22 \pm 2^\circ\text{C}$, and 55% relative humidity, with adequate ventilation. The animals were fed standard pellet chow recommended by the Animal Feed Production Center (Behparvar Company, registration number: 28100), and water was provided freely via 500 mL with adequate bottles.

Endurance Training Protocol

The exercise familiarization program consisted of five

sessions of walking and running on a treadmill at a speed of 5–8 m/min with zero incline for 8–10 minutes. Following familiarization, the exercise protocol included eight weeks of treadmill running, five days per week, with zero incline. Table 1 presents the training protocol for the rats. To determine individual maximum running speed, the rats initially ran on the treadmill at 5 m/min, and the speed was increased by 5 m/min every three minutes until the rats reached exhaustion. The final speed achieved was considered the maximum running speed, serving as an indicator of maximal oxygen consumption ($\text{VO}_{2\text{max}}$) to determine exercise intensity throughout the study (11).

Capsaicin Consumption

Capsaicin was administered at dosage of 5 mg/kg body weight, five days per week, for eight weeks via oral gavage, performed daily before noon (12). The dosage was adjusted weekly for each rat based on individual body weight. The capsaicin was dissolved in 0.5% carboxymethylcellulose solution to achieve a consistent concentration, allowing oral gavage administration at a fixed volume (1 mL/100 g body weight).

$\text{VO}_{2\text{max}}$ Measurement

pPst-test $\text{VO}_{2\text{max}}$ was measured using a rodent-specific digital treadmill with zero incline. Then the rat started running on the treadmill at 10 m/min. The speed was then increased by 3 m/min every three minutes until the rat were exhausted. The speed of the rat at the point of exhaustion was recorded as the rat's $\text{VO}_{2\text{max}}$ (11).

Tissue Sampling and Biochemical Analysis

Twenty-four hours after the final exercise session and capsaicin administration, following a 10–12 hour overnight fast, the rats were anesthetized via intraperitoneal injection of a mixture of xylazine (10 mg/kg) and ketamine (75 mg/kg). To measure calcium levels, approximately 5 mL of blood was directly collected from the abdominal aorta. Blood samples were centrifuged at approximately 3000 rpm for 15 minutes (Hettich EBA 20 centrifuge) and stored at -70°C until analysis. Serum calcium levels were measured using a spectrophotometric assay kit (Zist Shimi Company, Iran), with a measurement range of 2–20 mg/dL.

Real-Time Polymerase Chain Reaction Analysis

Following blood collection, the quadriceps muscles of the rats were excised, rinsed with physiological saline, and immersed in 1.8 mL microtubes containing 20% Ribonucleic acid (RNA)-later solution for stabilization before genetic analysis. Total RNA was extracted from the muscle tissue using the GeneX Trizol protocol according to the manufacturer's instructions. RNA quality and concentration were assessed, after which complementary Deoxyribonucleic acid (cDNA) was synthesized for reverse transcription.

ESR1 gene expression was measured using quantitative

Table 1. The Specific Training Protocol Parameters

Week	Time	Intensity (Maximum Speed)
1	10 min	40%
2	10 min	50%
3	15 min	50%
4	20 min	60%
5	25 min	65%
6	25 min	70%
7	30 min	75%
8	30 min	80%

Real-Time Polymerase Chain Reaction (PCR) (SYBR Green method), with *Glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) serving as the housekeeping control gene. Primer sequences and the lengths of the amplified fragments are listed in Table 2. All primers were designed spanning exon–exon junctions using Allele ID v7.8 software. To exclude potential genomic DNA contamination, 25 ng of cDNA and 25 ng of RNA were analyzed separately via 1.5% agarose gel electrophoresis. Amplification in the cDNA samples, observation of the expected band, along with no amplification in the RNA samples, confirmed the absence of genomic DNA.

PCR reactions followed standard Real-Time PCR procedures. Primer efficiency was assessed for each gene, and standard curves were generated accordingly. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method, with all reactions performed in triplicate to ensure reproducibility.

Data Analysis

Data are expressed as mean $\pm$ standard deviation (SD). The normality of data distribution and the homogeneity of variance were assessed using the Kolmogorov–Smirnov and Levene's test, respectively. Differences among the four groups were analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. The Statistical significance level was set at $P \leq 0.05$. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) (Win software, version 25).

Results

One-way ANOVA demonstrated significant differences in

VO₂max among the groups. Tukey's post hoc test indicated that VO₂max was significantly increased in the Exercise group ($P = 0.028$) and the Combined Exercise + Capsaicin group ($P = 0.004$) compared with the Control group. No significant difference in VO₂max was observed in the Capsaicin-only group compared to the Control group ($P = 0.12$), as depicted in Table 3.

One-way ANOVA showed significant differences in *ESR1* gene expression among the groups. Post hoc comparisons revealed that *ESR1* expression was significantly increased in the Exercise group ($P = 0.001$), the Capsaicin group ($P = 0.001$), and the Combined Exercise + Capsaicin group ($P = 0.033$) compared with the Control group. Notably, the increase observed in the Combined group was greater than that observed in either the Exercise or Capsaicin groups alone (Figure 1).

One-way ANOVA revealed a significant difference in serum calcium levels among the groups. Tukey's post hoc test showed that serum calcium levels were significantly higher in the Capsaicin group ($P = 0.001$) and the Combined Exercise + Capsaicin group ($P = 0.032$) compared with the Control group. Although calcium levels were higher in the Exercise-only group, this increase was not statistically significant ($P = 0.178$), as illustrated in Table 3.

Discussion

The results of the present study revealed that eight

Table 2. Primer Sequences and Corresponding Gene Fragment Lengths Used for Real-Time PCR

Genes Name	Primer Sequences	Accession Number
Rattus Norvegicus ESR1, Messenger RNA(mRNA)	Forward: ACCGAGTCTGGACAAGATCAAC	NM_012689.1
	Reverse: AAGACGATGAGCATCCAGCAT	
Rattus Norvegicus GAPDH	Forward: CAAGTTCAACGGCAGCTCA	NM_017008.4
	Reverse: CCCCATTTGATGTTAGCGGG	

Note. PCR: Polymerase chain reaction; ESR1: Estrogen receptor 1; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

Table 3. Comparison of ESR1 Expression, Calcium, and VO₂max Among Groups

Groups		Control	Exercise	Capsaicin	Exercise+ Capsaicin	Covariance P-value
ESR1 (gene expression)	Post-test Mean $\pm$ SD	0.29 $\pm$.087	1.53 $\pm$.021	1.40 $\pm$.013	2.06 $\pm$.037	0.033*
Post hoc (Tukey)	Post-test	-	↑ vs. Control	↑ vs. Control	↑ vs. Control, ↑ vs. Exercise, ↑ vs. Capsaicin	
Calcium (mmol/L)	post-test Mean $\pm$ SD	1.36 $\pm$.11	1.47 $\pm$.23	2.62 $\pm$.28	2.20 $\pm$.19	0.032*
Post hoc (Tukey)	Post-test	-	ns vs. Control	↑ vs. Control, ↑ vs. Exercise	↑ vs. Control ↑ vs. Exercise	
Vo ₂ max m/min	post-test Mean $\pm$ SD	20.14 $\pm$ 3.2	26.54 $\pm$ 2.87	21.37 $\pm$ 2.31	28.16 $\pm$ 3.47	0.004*

Note. ESR1: Estrogen receptor 1; VO₂max: Maximal oxygen consumption; ANOVA: Analysis of variance; SD: Standard deviation; mmol/L: Millimoles per liter; m/min: Meters per minute; ns: Not significant; *: Significant difference at $P \leq 0.05$; ↑: Significant increase compared to the specified group

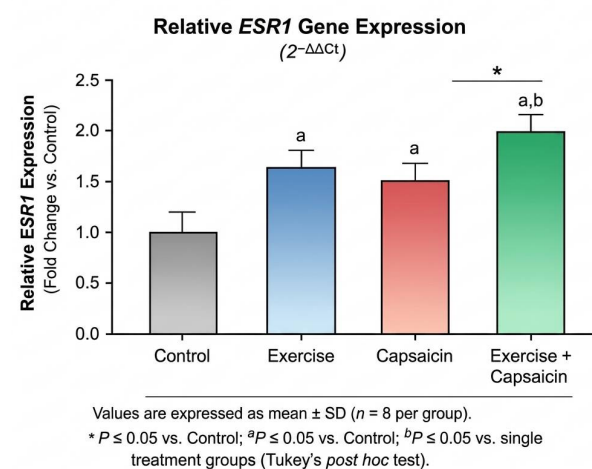


Figure 1. Relative ESR1 Expression in Control, Exercise, Capsaicin, and Combined Groups
Note. ESR1: Estrogen receptor 1

weeks of aerobic exercise and capsaicin supplementation significantly increased *ESR1* gene expression in the skeletal muscle of aged rats, with the most pronounced effects observed when these interventions are combined. These findings suggest that both aerobic training and capsaicin may enhance estrogen receptor-mediated signaling, which plays a key role in muscle function, metabolic regulation, and physiological adaptation during the aging process.

Estrogen receptors, particularly ER α , function as transcription factors that regulate genes involved in cellular growth, differentiation, and metabolic homeostasis (5). Age-related reductions in estrogen signaling and alterations in *ESR1* expression are closely associated with impaired muscle regeneration and metabolic dysfunction (13). In this regard, satellite cells, which are essential for muscle repair, are influenced by estrogen signaling, and decreased estrogen levels may reduce both their number and activity (14, 15). Therefore, the upregulation of *ESR1* expression may indicate an improved regenerative capacity and cellular function within the skeletal muscle of aged subjects.

Consistent with the present findings, previous studies have indicated that endurance exercise can modulate estrogen receptor expression, although these effects are often tissue-specific and influenced by exercise intensity and duration (8, 16). For example, Hao et al reported differential changes in estrogen receptor expression across muscle types following endurance training (16). Similarly, Malandish et al demonstrated increased ER α gene expression following aerobic exercise in older individuals (17). In addition, capsaicin has been reported to influence intracellular signaling pathways, particularly calcium-dependent mechanisms and metabolic regulators, which may indirectly modulate *ESR1* gene expression (18). The greater increase observed in the combined group suggests a potential synergistic effect between physical activity and capsaicin supplementation. However, some studies have reported contradictory findings, which may be attributed to differences in experimental models, hormonal status, or specific intervention protocols (19, 20).

Another significant finding of the present study was the increase in serum calcium levels in the capsaicin-supplemented and combined groups, whereas the exercise-only group did not exhibit a statistically significant change. Calcium is fundamental to muscle contraction, enzymatic activity, and bone metabolism (21). Capsaicin may influence calcium regulation by enhancing intestinal absorption and modulating relevant hormonal axes (22). In contrast, the effects of exercise on calcium balance and bone metabolism are highly dependent on training characteristics (23). Consequently, observed inconsistencies with other studies may be attributed to variations in study design and population characteristics (17, 24).

Conclusion

This study demonstrated that aerobic exercise combined with capsaicin supplementation significantly enhances *ESR1* gene expression in skeletal muscle of aged rats and may contribute to improved calcium regulation. These

findings suggest that combined lifestyle and nutritional interventions can positively influence the molecular and metabolic adaptations associated with aging. However, the precise underlying mechanisms of these effects are not yet fully understood. The lack of detailed endocrine assessments, ionized calcium measurements, and direct muscle functional or morphological evaluations should be considered when interpreting these findings. Further research is recommended to investigate hormonal regulators of calcium homeostasis, muscle anabolic signaling pathways, and the effects of different exercise intensities and capsaicin dosages to clarify the synergistic mechanisms involved.

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Artificial Intelligence Use Disclosure

None.

Authors' Contribution

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Competing Interests

The authors declare no conflict of interests.

Ethical Approval

This article was extracted from a Master's thesis submitted to Islamic Azad University, East Tehran Branch (Approval Code: IR.IAU.ET.REC.1403.051).

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