Research Article

# Impact of water resistance training and dark chocolate on IGF-1 and FOXO3 gene expressions in elderly women: Correlation analysis of variables

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## Abstract

Resistance exercises and nutritional strategy are the best ways to prevent with age- related muscle atrophy (sarcopenia) by improving the physiological function of skeletal muscle. This study investigated the effect of 8 weeks of Water resistance training with consumption of dark chocolate on the changes and correlation of IGF-1 and FOXO3 in elderly women. In this study, 40 sedentary elderly women with an age range of 60-73 years were randomly divided into four groups: 1. water resistance training, 2. water resistance training + dark chocolate, 3. dark chocolate, 4. control. The subjects of the training groups did water resistance training for eight weeks. During these 8 weeks, the dark chocolate groups consumed 30 grams of 83% dark chocolate every day, and the control group received neither exercise nor Dark chocolate. Blood sampling was done 48 hours before the study and after the end of the last training session. Expressions of IGF-1 and FOXO3 were measured by real time-PCR method. After 8 weeks of water resistance training and consumption of dark chocolate, the expression of insulin-like growth factor 1 (IGF-1) gene increased significantly compared to the pretest ($p<0.05$). However, FOXO3 did not change significantly ($p>0.05$). These findings underscore the potential of targeted exercise and dietary interventions in enhancing muscle health among the elderly, although further research is needed to understand the implications of unchanged FOXO3 in this context.

***Key Words:*** **Water resistance training, Dark chocolate, Elderly, IGF-1, FOXO3**

## Introduction

The global population is rapidly aging, and preventing or delaying age-related disabilities is an important public health issue (Espeland et al., 2007). Aging was associated with a progressive decline in muscle mass and physical performance. Sarcopenia is defined as the loss of skeletal muscle mass, muscle strength, and physical function associated with aging. This phenomenon is linked to adverse health outcomes and a decrease in quality of life (Jentoft-Cruz et al., 2010). Sarcopenia is a complex, multifactorial condition, and its onset and progression depend on various factors, including physical inactivity, neuromuscular dysfunction, hormonal changes, genetic factors, and inadequate nutrition (Jentoft-Cruz et al., 2010; Liguori et al., 2018; Rolland et al., 2008; Ziaaldini et al., 2017). In recent years, extensive research has been conducted to clarify the cellular and molecular mechanisms underlying muscle hypertrophy and atrophy. It is believed that the onset of age-related sarcopenia is due to one or more factors, including an increased rate of muscle protein breakdown, decreased muscle protein synthesis, or a combination of these factors (Wilkinson et al., 2018). The reduction of IGF-1, as an anabolic factor, predisposes elderly individuals to sarcopenia, cardiovascular diseases, and functional dependency as a result of aging (Lang et al., 2010; Juul et al., 2002; Gibney et al., 2007).

Studies have shown that higher serum levels of IGF-1 are associated with better muscle mass and strength in older populations. For example, one study found that elevated serum IGF-1 levels were linked to a lower prevalence of sarcopenia, particularly in older men, indicating a protective effect against muscle loss (Jiang et al., 2022). Conversely, low levels of IGF-1 are associated with an increased risk of sarcopenia, highlighting its potential as a biomarker for assessing muscle health in older adults (Bian et al., 2020). Therefore, IGF-1 may have a significant role in the management of sarcopenia. Among the many regulatory genes in the skeletal muscle system, the Forkhead box O3 (FOXO3) protein is central due to its inactivation during muscle growth. Therefore, inhibiting

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FOXO3 may be a potential target in the treatment of muscle atrophy (Schiaffino et al., 2013). Targeting the FOXO3 signaling pathway could provide new strategies for the treatment of sarcopenia. Interventions that increase FOXO3 activity or modulate its downstream effects may be beneficial. It may help preserve muscle mass and function in the elderly.

For example, compounds that activate the mTOR/Akt pathway can lead to increased phosphorylation of FOXO3, thereby reducing its activity and potentially counteracting muscle loss (Oh et al., 2023; Gellhaus et al., 2023). FOXO3, as a downstream member of the IGF-1 signaling pathway, plays an important role in protein degradation and the anabolic/catabolic protein balance in the musculoskeletal system. There is significant crosstalk between the PI3K/AKT signaling pathway and IGF-1 and FOXO3 (Schiaffino et al., 2013).

IGF-1 plays an important role in cellular growth, metabolism, and survival. The interaction between IGF-1 and FOXO3 is particularly significant in areas such as muscle health. High levels of IGF-1 are associated with muscle hypertrophy and reduced atrophy, while low levels can lead to increased activity of FOXO3 and subsequent muscle wasting (Li et al., 2020). Both of these factors can be influenced by exercise and nutritional supplements. Resistance training is considered one of the important indicators of physical fitness for individuals of all ages in society. Resistance training helps to maintain and increase muscle mass and improves muscle strength. It also leads to increased bone density, reduced blood pressure, decreased body fat, and improved motor functions (Kraemer et al., 2002). Previous research has also shown that resistance training can be effective in preventing muscle atrophy and reducing muscle mass and strength (Bae et al., 2020; Moradi, 2021). Hyun Bi et al. (2021) demonstrated in their study that, compared to the control group, the levels of factors related to autophagy and FOXO3 significantly decreased in the quadriceps and soleus muscles of the resistance training group. Additionally, resistance training significantly increased the phosphorylation of FOXO3 via protein kinase B (AKT) (Bae et al., 2021). In addition to exercise, the consumption of dark chocolate also affects these factors. Dark chocolate is rich in flavonoids, which have antioxidant properties that may influence cellular pathways, including those related to FOXO3. Antioxidants help reduce oxidative stress, a factor that can impact FOXO3 activity and its role in muscle preservation and quality (Nemecz, 2005). Dark chocolate is associated with improved insulin sensitivity and reduced blood pressure, factors that can indirectly affect IGF-1 levels. IGF-1 is crucial for muscle growth and development, and its regulation is essential for maintaining muscle mass during aging (Grassi et al., 2005). Considering the beneficial effects of exercise and dark chocolate, we hypothesize that both water resistance training and dark chocolate will positively influence IGF-1 gene while modulating FOXO3 gene in elderly women, potentially contributing to improved health outcomes.

## Materials and Methods

### Subjects

In this semi-experimental study, after announcing the call for participants, 40 healthy elderly women were selected using purposive and convenient sampling methods. The samples in this research were aged between 60 and 73 years. The inclusion criteria for the study included: healthy elderly individuals aged between 60 and 73 years, sedentary, and not participating in regular physical activities in the past 12 months. The exclusion criteria included: a history of specific diseases (chronic pulmonary disease, heart disease, and hypertrophic heart failure), smoking; not taking any supplements or medications in the past month, mental health issues (such as dementia), and musculoskeletal or joint injuries. All participants completed a consent form and participated with full awareness of the study's objectives, and their information remained completely anonymous in accordance with the ethical protocols of the Helsinki studies. The proposal was registered with the approval of the Research Vice Presidency and the Specialized Ethics Committee in Biomedical Research at Islamic Azad University, Science and Research Branch, Tehran, with the ethics code 1403.088REC.SRB.IAU.IR (IRCT: 20240519061840N1).

"The participants completed the PARQ (Physical Activity Readiness Questionnaire) to be screened for participation in this research by a physician. Among the 40 selected participants in this study, 3 were excluded due to non-cooperation, personal issues, and illness during the research. Therefore, the study was ultimately continued with 37 individuals. Before starting the 8-week intervention, information regarding age, height, weight, and body mass index (BMI) was recorded. For assessing body composition, the BODY COMPOSITION device made by Omron, model BF 511, was used, and for measuring height, a Seca stadiometer with an accuracy of 0.1 centimeters was employed."

### Grouping

The participants were randomly assigned to four groups: 1) water resistance training (8 individuals), 2) water resistance training + Dark chocolate (9 individuals), 3) Dark chocolate (10 individuals), and 4) control (10 individuals, maintained their usual dietary habits and physical activity levels). The group undergoing water resistance training participated in eight weeks' water resistance training and did not consume any Dark chocolate. The group participating in water resistance training with Dark chocolate received 30 grams of 83% Dark chocolate daily during the eight weeks of water resistance training. The Dark chocolate group consumed the specified supplement at the same dosage daily for

eight weeks but did not participate in any exercise. The control group received neither exercise nor dark chocolate.

### Water resistance training protocol

The participants in the training groups performed the selected training program in the pool three non-consecutive days a week for eight weeks. Each training session included a warm-up and mobility exercises for 10 to 15 minutes, followed by weight training consisting of four upper body exercises: shoulder abduction and adduction, shoulder flexion and extension, elbow flexion and extension, and elbow flexion with a push-up board. Additionally, there were four lower body exercises: hip abduction and adduction, hip extension, triple hip flexion, and knee and ankle joint flexion, for a duration of 45 minutes. Finally, a cool-down period of 5 to 10 minutes was conducted with stretching exercises (Mousavi & Ghazalian, 2020)

### Laboratory measurements

Before and after the start of the eight-week program, blood samples were evaluated, with participants fasting for 12 hours. Blood was drawn from the left arm vein while seated, in two instances: 48 hours before the first training session and 48 hours after the last training session. To assess changes in the expression of factors IGF-1 and FOXO3 genes, blood samples were collected in tubes containing EDTA and transferred to the laboratory. The blood samples were stored at -70 degrees Celsius and measured using the Real-Time PCR method.

### RNA extraction process

Real-time PCR is a highly sensitive technique that allows for the quantification of mRNA expression, providing insight into the transcriptional regulation of IGF-1 and FOXO3. By measuring mRNA expression, we can assess how water resistance training and dark chocolate may influence the transcriptional activity of these genes. In addition, Real-time PCR provides greater sensitivity compared to traditional methods, enabling the detection of low abundance transcripts that might not be captured by ELISA alone. This is particularly relevant in elderly populations where variations in gene expression can be subtle. Therefore, we use RT Pcr method.

To extract RNA from blood (peripheral blood mononuclear cell), the cDNA extraction kit from Pars Tos, made in Iran, was used. First, according to the kit instructions, 200 µl of blood sample was placed into a microtube, and 1 milliliter of Trizol was added. After thorough mixing (pipetting), it was incubated at room temperature for 5 minutes. Then, 200 µl of cold chloroform was added, and after pipetting (for 15 seconds), it was incubated at room temperature for about 2 to 3 minutes. Next, the microtubes were centrifuged at 13,000 RPM for 12 minutes at 4 degrees Celsius.

The supernatant was carefully removed using a pipette and transferred to a RNase-free microtube. Then, 1.5 milliliters of cold isopropanol was added, and the microtube was centrifuged at 12,000 RPM for 10 minutes at 4 degrees Celsius.

Again, at this stage, a white precipitate was visible at the bottom of the microtube. The supernatant was carefully removed using a pipette, and 1 milliliter of cold ethanol was added. After a brief shake, it was centrifuged at 13,000 RPM for 1 minute at 4 degrees Celsius. Next, the supernatant was carefully drained, and the microtube was placed open under the hood for 10 minutes to allow the remaining ethanol to evaporate and to dry the inside of the microtube. After this step, 35 µl of injectable water was added to the sample, and gentle pipetting was performed several times. Finally, the concentration and purity of the extracted RNA were assessed at wavelengths of 260 and 280 nanometers, and its concentration was determined based on the dilution factor in ng/µl.

### cDNA synthesis

To transcribe RNA into cDNA, the cDNA extraction kit from Pars Tos, made in Iran, was used. First, according to the kit instructions, a specific amount of RNA from each sample, reaction buffer, dNTP mix, random hexamer, master mix, and distilled water were added and combined in a microtube for each sample. Then, for the transcription to cDNA, the thermocycler was programmed as follows: incubation at 25 degrees Celsius for 10 minutes, followed by 60 minutes at 42 degrees Celsius, and finally, an increase in temperature to 85 degrees Celsius for 5 minutes. All steps were performed according to the manufacturer's instructions. At the end, the samples were transferred to -20 degrees Celsius for gene expression analysis.

### Gene expression

To assess gene expression, the Real-time PCR method was used. In this study, the amplification of the IGF-1 and FOXO3 genes, as well as the reference gene (GAPDH), was performed using Real-time PCR based on the standard method. To measure the decrease or increase in the expression of the IGF-1 and FOXO3 genes, their expression was compared with that of internal control genes. For this purpose, 10 microliters of the master mix were taken, and 1 microliter of each primer and 7 microliters of water were added. These volumes were placed in 8-strip tubes, with 8 microliters in each strip. Then, 2 microliters of cDNA were added to each strip, resulting in a final reaction volume of 20 microliters for Real-time PCR. The Real-time PCR process was carried out using the Corbett Gene-ROTOR model 6000 thermocycler, and each reaction was performed in duplicate. After the reaction and data extraction, the calculation of gene expression was performed using the $2^{-\Delta\Delta_{Ct}}$ (fold change) method.

### Primer design and gene expression determination

First, the mRNA sequences corresponding to the IGF-1 and FOXO3 genes were extracted using the NCBI website. Primers were designed using the Runner Gen software, and then each primer was evaluated using the BLAST software to ensure the uniqueness of the primer binding sites (Table 1). The primers were synthesized by the Sinaclon company. In this study, the GAPDH gene was used as an internal control.

#### Statistical analysis

For data analysis, the normality of the data distribution and the homogeneity of variances were assessed using the Shapiro-Wilk and Levene tests. Then, a one-way ANOVA was used to compare intergroup variations, followed by the Bonferroni post hoc test to determine the exact location of these differences at a significance level of ($P \geq 0.05$). Additionally, Pearson correlation was used to examine the relationship between FOXO3 and IGF-1.

## Results

### Anthropometric measurement

In this study, in order to quantitatively describe the variables of the participants' population, the age, height, weight and body mass index of the four groups were measured (Table 2).

### Gene expression of FOXO3

Serum FOXO3 gene expression in different research groups is shown in Figure 1. According to the results of ANOVA statistical test, there is no significant difference in serum FOXO3 gene expression between different research groups ($F=0.288$, $p=0.833$).

### Gene expression of IGF-1

Serum IGF-1 gene expression in different research groups is shown in Figure 2. Based on the results of ANOVA statistical test, there is a significant difference in serum IGF-1 gene expression between different research groups ($F=12.60$, $p \leq 0.0001$).

**Tale 1.** Primer sequence of the IGF-1 and FOXO3

| Genes | Primer Sequence (5'->3') |
|---|---|
| IGF-1-For | 5-GTGGATGCTCTTCAGTTCGTGTG-3 |
| IGF-1-Rev | 5-TCCAGTCTCCTCAGATCACAGC-3 |
| FOXO3-For | 5-CCTACTTCAAGGATAAGGGCGAC-3 |
| FOXO3-Rev | 5-GCCTTCATTCTGAACGCGCATG-3 |
| GAPDH-For | 5-CATGGCCTCCAAGGAGTAAGA-3 |
| GAPDH-Rev | 5-GAGGGAGATGCTCAGTGTTGG-3 |

Tukey,s post hoc test results showed that Exe and Exe+Sup groups had a significant increase in the IGF-1 gene expression compared to Con and Sup groups ($p<0.05$).

### Correlation

Pearson's correlation was used to investigate the relationship between IGF-1 and FOXO3 genes (Figure 3). The results of this test showed that there is no correlation between IGF-1 and

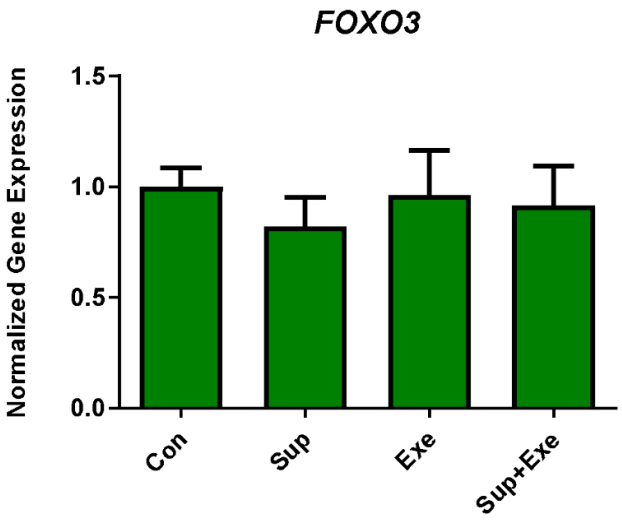


**Figure 1.** Gene expression of FOXO3 at different groups of study. The data were show as mean ± standard division ($p<0.05$). Con: Control, Sup: Supplement, Exe: Exercise

**Table 2.** Anthropometric measurement of subjects in different groups.

| Variable | Con | Sup | Exe | Sup+Exe | F | Sig |
|---|---|---|---|---|---|---|
| Number | 10 | 10 | 8 | 9 | - | - |
| Age | 63 ± 1.66 | 67.3 ± 2.8 | 64.2 ± 2.7 | 64.8 ± 3.6 | 1.66 | 0.193 |
| Hight (cm) | 156.8 ± 4.0 | 158.6 ± 3.6 | 156.1 ± 5.3 | 157.3 ± 4.8 | 0.532 | 0.663 |
| Weight (kg) | 71.3 ± 7.7 | 73.1 ± 8.6 | 71.6 ± 7.2 | 71 ± 7.5 | 0.137 | 0.937 |
| BMI($m^2$) | 29 ± 3.1 | 29 ± 3.5 | 29.3 ± 2.7 | 28.3 ± 2.3 | 0.491 | 0.691 |

The data were show as mean ± SD (p<0.05). Con: Control, Sup: Supplement, Exe: Exercise.

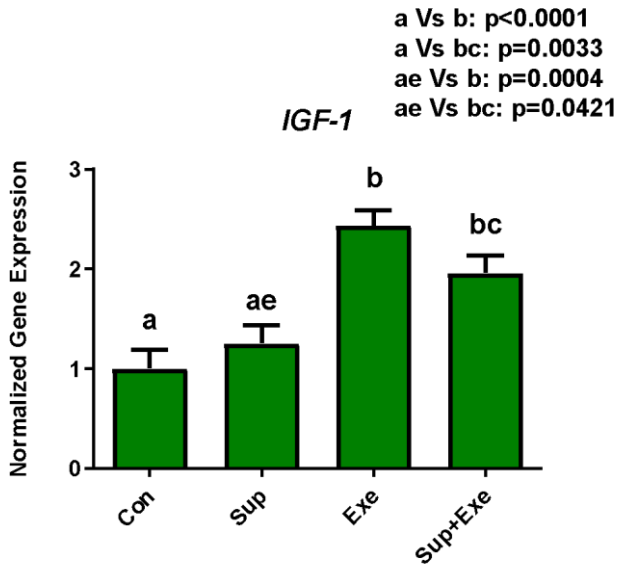


**Figure 2.** Gene expression of IGF-1 at different groups of study. The data were show as mean ± standard division (p<0.05). Con: Control, Sup: Supplement, Exe: Exercise

FOXO3 genes in different research groups (p>0.05).

## Discussion

Aging profoundly affects sarcopenia markers through various mechanisms, including loss of muscle mass and strength, changes in muscle fiber composition, alterations in biomarkers of inflammatory and oxidative stress, and a reduction in regenerative capacity. Understanding these changes is crucial for developing strategies to mitigate the impact of sarcopenia on the elderly population. Additionally, controlling sarcopenia and its markers at the cellular level can have therapeutic implications. Therefore, the present study examines the effects of 8 weeks of resistance training in water, combined with the consumption of dark chocolate, on the changes and correlations of sarcopenia markers IGF-1 and FOXO3 in elderly women.

The results of the present study indicated that the expression of the FOXO3 gene did not show significant changes in the different groups after 8 weeks of resistance training in water and dark chocolate consumption. FOXO3 is a transcription factor involved in regulating muscle atrophy and metabolism. Our investigation

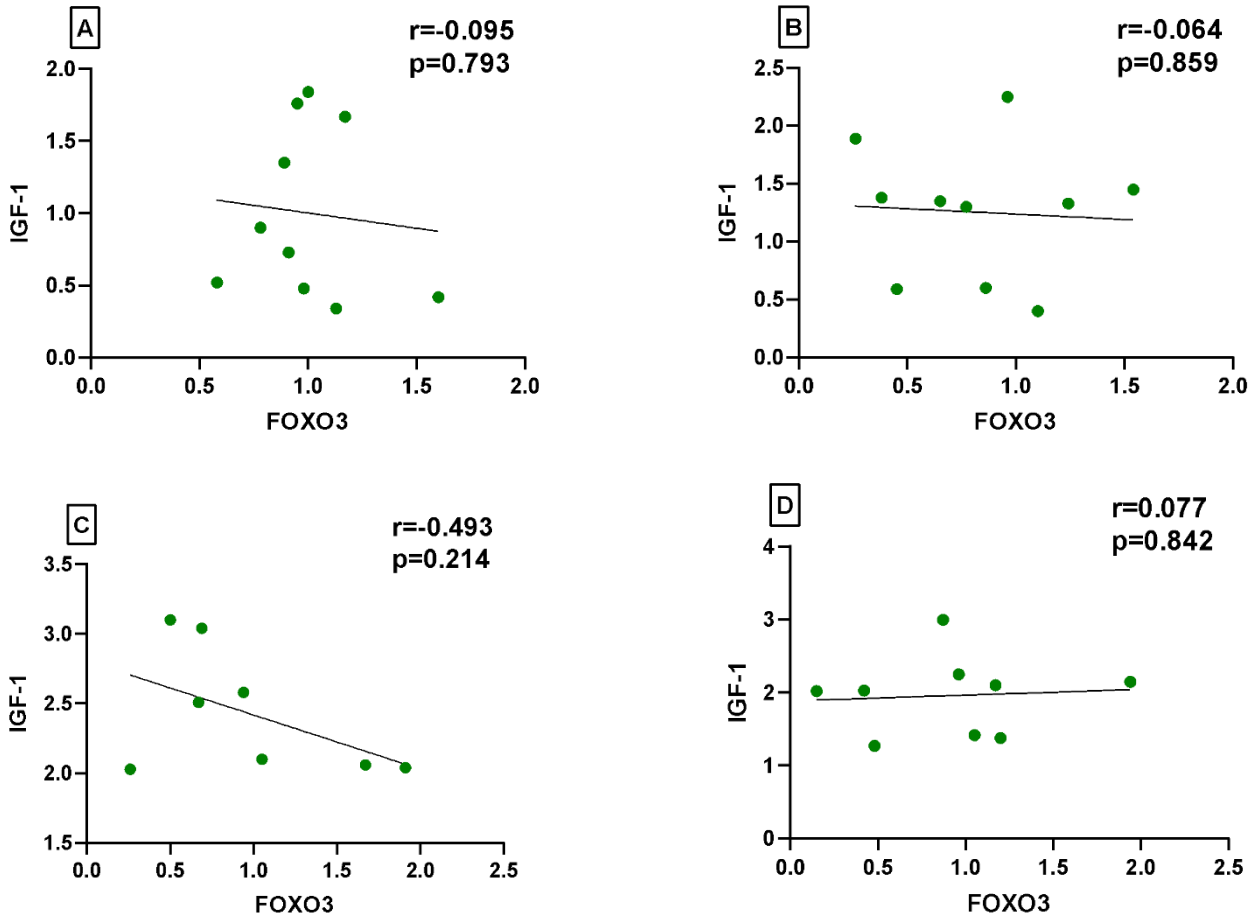


Figure 3. Pierson correlation between IGF-1 and FOXO3 at different groups (A: control, B: supplement, C: exercise, D: supplement+exercise). The data were show as mean ± standard division (p<0.05). Con: Control, Sup: Supplement, Exe: Exercise

into FOXO3 gene alongside IGF-1 provides a broader understanding of the molecular mechanisms at play. Previous research indicates that increased FOXO3 activity is linked to muscle degradation, while enhanced IGF-1 signaling can counteract this effect (Bian et al., 2020). Thus, our results may reveal a complex interplay between these factors during resistance training. In contrast, the study by Sadri et al. (2020) demonstrated that 8 weeks of HIIT (High-Intensity Interval Training) in both upright and supine positions resulted in a significant reduction in FOXO3 gene expression in the gastrocnemius muscle of male Wistar rats. However, in the present study, we used human subjects and also employed water-based resistance training, which may account for the differences in results due to variations in the samples used, the type of exercise, and the intensity of the training. Since water-based resistance exercises, especially in elderly women, do not impose significant stress on the body, and the water environment reduces the force exerted on the muscles, it appears that this type of training may have a lesser role in negatively regulating autophagy and sarcopenia indicators in muscle. Multiple mechanisms may contribute to the non-significant findings regarding FOXO3. One possibility is that eight weeks may not be sufficient to induce measurable changes in FOXO3 genes. Longer intervention periods may be necessary to observe significant physiological adaptations. Additionally, the elderly population is highly heterogeneous due to variations in baseline fitness levels, nutritional status, and metabolic responses, which can affect the efficacy of the intervention. On the other hand, while dark chocolate is recognized for its antioxidant properties, its impact on FOXO3 may be influenced by dosage or bioavailability (Bian et al., 2020)

While our findings indicated no significant change in FOXO3 gene, it is essential to contextualize this result within the broader framework of sarcopenia. FOXO3 is known to play a dual role in muscle homeostasis: it regulates both muscle growth and degradation pathways. Specifically, FOXO3 activation leads to increased expression of genes involved in protein degradation, such as Atrogin-1, which is implicated in muscle atrophy (Li et al., 2020). The lack of significant change in FOXO3 gene may suggest that while IGF-1 is promoting anabolic processes, the regulatory mechanisms involving FOXO3 remain stable, potentially indicating a balance between anabolic and catabolic signaling pathways. This could imply that our intervention effectively enhances muscle-building signals without triggering excessive degradation pathways.

In contrast to the results for FOXO3, the expression of the IGF-1 gene showed a significant increase in the exercise groups as well as in the exercise + dark chocolate group. Numerous studies have established a negative correlation between serum IGF-1 gene and sarcopenia. For instance, a study involving subjects with type 1 diabetes mellitus found that lower serum IGF-1 gene were significantly associated with sarcopenia and reduced skeletal muscle mass (Hata et al., 2021). Similarly, research on elderly patients undergoing hemodialysis indicated that lower IGF-1 gene correlated with increased sarcopenia prevalence (Widajanti et al., 2022). This aligns with our findings, where we observed variations in IGF-1 gene in response to our interventions. Resistance training has been shown to enhance IGF-1 signaling pathways, which are crucial for muscle hypertrophy and regeneration. A study utilizing Mendelian randomization analysis demonstrated that elevated IGF-1 gene are associated with reduced risk of low hand grip strength and increased lean mass (Liu et al., 2024). Our findings suggest that water resistance training may similarly stimulate IGF-1 production, supporting muscle maintenance and growth in elderly women. This is consistent with the notion that physical activity can positively influence anabolic hormones like IGF-1. Shafei et al. (2021) examined the effects of eight weeks of water cycling versus land cycling on serum testosterone and IGF-1 gene in elderly men and found that the water cycling program resulted in an increase in testosterone and IGF-1 gene, which aligns with the results of the present study. Water-based resistance training engages multiple muscle groups and promotes muscle hypertrophy. This type of exercise can stimulate the secretion of growth hormones, including IGF-1, which play a crucial role in muscle repair and growth. Studies have shown that resistance training can lead to a significant increase in IGF-1 gene. Although these changes may not always persist in the long term (Gulick et al., 2020), dark chocolate is rich in flavonoids, which have been shown to improve endothelial function and increase nitric oxide availability. This effect can enhance blood flow and potentially boost the delivery of essential nutrients for recovery and muscle growth, indirectly affecting IGF-1 gene (Grassi et al., 2005). Considering the effects of exercise and dark chocolate, it appears that these two factors may have synergistic effects in increasing IGF-1 gene. It has been stated that increasing IGF-1 gene aligns with existing literature that highlights the role of IGF-1 as a crucial anabolic factor in muscle metabolism. Increased IGF-1 gene are associated with enhanced muscle protein synthesis and may counteract the effects of sarcopenia, which is characterized by a decline in muscle mass and strength in older adults (Zhao & Liu, 2021). The elevation of IGF-1 suggests that water resistance training, combined with dark chocolate consumption, could be a viable strategy to mitigate muscle atrophy associated with aging.

IGF-1 is a potent anabolic factor that promotes muscle protein synthesis through the activation of the Akt/mTOR pathway. Upon binding to its receptor, IGF-1 activates the phosphatidylinositol 3-kinase (PI3K) pathway, leading to the phosphorylation of Akt (also known as Protein Kinase B). This cascade results in several key outcomes: Akt phosphorylates and activates the mechanistic target of rapamycin complex 1 (mTORC1), which is essential for

stimulating protein synthesis by promoting ribosomal biogenesis and translation processes. This activation enhances the synthesis of myofibrillar proteins, crucial for muscle hypertrophy and repair (Yoshida & Delafontaine, 2020).

Concurrently, IGF-1 signaling inhibits pathways that lead to muscle atrophy. Specifically, it represses FOXO transcription factors (including FOXO3), which are known to promote the expression of ubiquitin ligases such as Atrogin-1 and MuRF-1. These ligases are responsible for targeting muscle proteins for degradation via the ubiquitin-proteasome system (Yoshida & Delafontaine, 2020). By inhibiting FOXO3 activity, IGF-1 reduces protein breakdown, thereby supporting muscle maintenance and growth.

There have been limited studies regarding the correlation and crosstalk between IGF-1 and FOXO3. However, given that these factors interact as signaling molecules, there is a relationship between them, particularly in the context of hypertrophy and sarcopenia. FOXO3 acts as a transcription factor that regulates various genes involved in apoptosis, cell cycle control, and resistance to oxidative stress. Therefore, the inhibition of FOXO3 by IGF-1 may influence these pathways, potentially leading to a decrease in the expression of genes that cause muscle atrophy while increasing the expression of genes that support cell survival (Santos et al., 2023). The balance between IGF-1 signaling and FOXO3 activity is particularly relevant in the context of aging and sarcopenia. A reduction in IGF-1 signaling or an increase in FOXO3 activity may contribute to muscle loss and decreased muscle function associated with aging, highlighting the importance of this interaction in maintaining muscle health in older adults (Jing et al., 2023). Nevertheless, in the present study, the correlational changes between these two factors were not confirmed. It seems that the serum changes of these factors are influenced by multiple factors; therefore, it would be better to evaluate these two factors in muscle tissue in future studies.

Also, our study underscores the importance of integrating exercise interventions such as water resistance training with dietary considerations like dark chocolate consumption. These strategies not only mitigate sarcopenia but also significantly enhance the quality of life for elderly women. Future research should focus on long-term effects and broader applications across diverse aging populations.

The sample size of our study was relatively small, which could limit the generalizability of our results. A larger sample may provide more robust data and allow for a better understanding of the effects of water resistance training and dark chocolate on IGF-1 and FOXO3 genes.

## Conclusion

The results suggest that increased IGF-1 may lead to a downreg-ulation of FOXO3 activity, thereby potentially mitigating the effects of sarcopenia—a condition characterized by muscle loss and functional decline in older adults. This interplay underscores the importance of maintaining optimal IGF-1 gene through targeted physical activity and dietary interventions, which may offer therapeutic avenues for addressing sarcopenia in elderly populations.

## What is already known on this subject?

Resistance exercises and nutritional strategy are the best ways to prevent with age- related muscle atrophy (sarcopenia) by improving the physiological function of skeletal muscle.

## What this study adds?

increased IGF-1 may lead to a downregulation of FOXO3 activity, thereby potentially mitigating the effects of sarcopenia—a condition characterized by muscle loss and functional decline in older adults.

**Organ Cross-Talk Tips:**

- Manipulating the interactions between FOXO3 and IGF-1 through targeted exercise regimens, individuals can better manage muscle health and combat atrophy effectively

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## Compliance with ethical standards

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

**Ethical approval** The proposal was registered with the approval of the Research Vice Presidency and the Specialized Ethics Committee in Biomedical Research at Islamic Azad University, Science and Research Branch, Tehran, with the ethics code 1403.088REC.SRB.IAU.IR (IRCT: 20240519061840N1).

**Informed consent** Not applicable

## Author contributions

Conceptualization: M.M., H.A., M.Gh.; Methodology: Sh.S., H.A., F.Gh.; Software: H.A.; Validation: M.M., Formal analysis; Investigation: M.Gh., F.Gh.; Resources: Sh.S., H.A.; Data curation: M.M., H.A., M.Gh.; Writing - original draft: Sh.S.; Writing – review & editing: H.A.; Visualization: M.Gh.; Supervision: H.A. Project administration: H.A.; Funding acquisition: M.M.

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