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Investigation of MuRF1 and Atrogin-1 gene expression changes in the soleus muscle of obese diabetic rats following 8 weeks of high-intensity interval training combined with quercetin supplementation

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Abstract

Background: MuRF1 and Atrogin-1 are two key genes involved in the muscle atrophy pathway and are upregulated in diabetes, particularly under conditions of insulin resistance and inflammation, leading to increased degradation of muscle proteins and reduced muscle mass. Therefore, this study aimed to investigate changes in MuRF1 and Atrogin-1 gene expression in the soleus muscle of obese diabetic rats following 8 weeks of high-intensity interval training (HIIT) combined with quercetin supplementation.

Methods: In this experimental study, 25 adult male rats (Age= 12 weeks old and weight= 180-200 gr) were purchased and randomly assigned to five groups: healthy control (HC, n= 5), diabetes control (DC, n= 5), quercetin control (QC, n= 5), HIIT (HIIT, n= 5), and HIIT with quercetin (QHIIT, n= 5). Eight weeks of HIIT training was performed. Type 2 diabetes (T2DM) was induced in the rats using STZ and 8 weeks of a high-fat diet. Quercetin was injected for 8 weeks in the supplementation groups. One-way analysis of variance with Tukey's post-hoc test was used to analyze the data. Significance was set at P-Value < 0.05.

Results: The results showed that after T2DM induction, blood glucose and MuRF1 and Atrogin-1 gene expression levels in the soleus muscle of rats significantly increased (P-Value<0.001). After eight weeks of training and supplementation interventions, blood glucose significantly decreased in all intervention groups (P-Value =0.001), and only in the HIIT with quercetin supplementation group was there a significant decrease in both MuRF1 and Atrogin-1 gene expression (P-Value =0.001).

Conclusion: HIIT and quercetin supplementation alone reduced blood glucose levels in diabetic rats. Although HIIT and quercetin supplementation alone did not affect MuRF1 and Atrogin-1 gene expression, the combination of both interventions resulted in significant changes. This suggests that the combined use of HIIT exercise and quercetin supplementation has a notable effect on selected factors related to atrophy in diabetic rats.

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Highlights

What is current knowledge?

Previous studies have shown that exercise training and quercetin supplementation may improve glucose metabolism, reduce inflammation and oxidative stress, and potentially influence pathways related to muscle atrophy.

What is new here?

- This study demonstrated that 8 weeks of combined HIIT and quercetin supplementation significantly reduced MuRF1 and Atrogin-1 gene expression in the soleus muscle of obese diabetic rats, indicating a protective effect against muscle atrophy.
- The study also showed that HIIT alone or quercetin supplementation alone lowered blood glucose but did not significantly alter MuRF1 and Atrogin-1 expression, suggesting that the beneficial effect on atrophy-related genes requires the combination of both interventions.

Introduction

According to the International Diabetes Federation (IDF), the number of adults with diabetes worldwide has reached 537 million, representing a significant increase of 74 million compared with 2019 (1). The future outlook is not more favorable, as the number of individuals with diabetes is expected to reach 643 million by 2030 (1). Despite technological advances in the development of new chemical agents for the treatment of diabetes mellitus (DM), the prevalence and complications associated with this disease continue to increase, indicating an urgent need for new treatment options with sufficient efficacy (2,3). Skeletal muscle constitutes approximately 40% of body weight and plays an important role in amino acid and glucose metabolism, protein storage, and locomotion. Therefore, maintaining muscle mass and function is essential for human health. Immobility, such as that occurring during casting, spaceflight, or bed rest, causes disuse-induced muscle atrophy (4). Musculoskeletal health consistently emerges as a common feature in the progression of these metabolic disorders (5). Skeletal muscle atrophy and dysfunction can further impair whole-body metabolism and reduce exercise capacity, thereby initiating a vicious cycle that worsens the underlying conditions (6). However, the myocellular consequences of these metabolic disorders remain incompletely understood.

Insulin signaling not only facilitates skeletal muscle glucose uptake but also plays a pivotal role in skeletal muscle anabolism, largely by suppressing catabolic pathways and facilitating the anabolic response to nutrient feeding (5). Among the markers involved in muscle atrophy, MuRF1 and Atrogin-1 are upregulated in several conditions of muscle atrophy and degrade proteins involved in muscle protein synthesis, remodeling, and contraction (7). In early studies in which Atrogin-1 or MuRF1 was deleted, rats were resistant to denervation-induced muscle atrophy (8). Although these experiments provided evidence for a mechanistic role of Atrogin-1 and MuRF1 in denervation-induced muscle wasting, few studies have examined the role of ubiquitin ligases in muscle loss under other conditions. In some of these studies, evidence indicated that Atrogin-1 is involved in muscle wasting induced by fasting or oxidative stress (9). In other reports, MuRF1 has been implicated in the loss of muscle mass and function induced by NF-κB and TNF-α activation (10).

The management of type 2 diabetes (T2DM) has changed over the past decade. In fact, the availability of new medications, improved monitoring devices, and a better understanding of the impact of exercise and dietary control in the treatment of this disease have changed its management, including the use of supplements for disease control. Among all types of exercise, high-intensity interval training (HIIT) has been widely recognized as a beneficial training method; however, the cellular and molecular effects of HIIT training that improve performance are not well understood. In today's world, the use of supplements for muscle building and enhancing the effectiveness of exercise training has been increasingly studied, including quercetin. Quercetin has significant anti-diabetic effects and may be beneficial in lowering blood glucose and increasing insulin sensitivity (11). In this regard, the results of one study showed that dietary quercetin administration for 14 days prevented disuse-induced muscle atrophy in SNX-operated GM rats. Quercetin activated phosphorylation of Akt, a key regulator of muscle synthesis in skeletal muscle, and increased plasma IGF-1. Quercetin administration tended to reduce lipid hydroperoxide production in atrophied muscle, suppress mitochondrial-induced hydrogen peroxide production, and protect mitochondria from the reduction in biogenesis induced by SNX surgery (4). Understanding the mechanisms involved in muscle atrophy can help develop new therapeutic approaches for atrophic conditions. Skeletal muscle atrophy can increase the expression of some genes related to muscle atrophy through biochemical and transcriptional pathways. However, regarding the effectiveness of HIIT training in diabetic patients, it has been reported that this type of training improves muscle function. Furthermore, HIIT prevents skeletal muscle atrophy, increases resistance to fatigue, and has an anti-inflammatory effect (12). Therefore, we hypothesized that the combination of HIIT and quercetin supplementation would exert a synergistic effect on reducing MuRF1 and Atrogin-1 gene expression in the soleus muscle of obese diabetic rats compared with either intervention alone. Since resistance training is more commonly used to prevent atrophy and aerobic training, particularly HIIT, has been less extensively studied, this study aimed to investigate changes in MuRF1 and Atrogin-1 gene expression in the soleus muscle of obese diabetic rats following 8 weeks of HIIT combined with quercetin supplementation.

Methods

The present experimental study used a post-test-controlled design. The animal samples consisted of 25 male Wistar rats (Weight: 180-200 gr, age: 12 weeks), which were purchased from the Animal Care Center of the University of Ardabil. The present experimental study was conducted in a laboratory setting with a post-test design, and its proposal was registered with the Research Ethics Committee of Mohaghegh Ardabili University under the number IR.UMA.REC.1402.042. After 8 weeks of a high-fat diet and familiarization with the laboratory environment and treadmill, diabetes was induced in 20 animals. To induce T2DM, a high-fat diet was used for 8 weeks, and then freshly prepared STZ solution (STZ: Sigma, St. Louis, MO), 25 mg/kg dissolved in citrate buffer with pH=5.4, was administered intraperitoneally. Non-diabetic rats were also injected with an equivalent volume of citrate buffer. To prepare high-fat diet (HFD), 1% cholesterol powder and 1% pure corn oil 100% were added to the standard food. One week after the STZ injection, a drop of blood was placed on a glucometer strip and measured using a glucometer, and rats with blood glucose levels between 200 and 400 mg/dL were considered diabetic samples. To ensure that blood glucose did not return to normal, the rats' blood glucose was also measured at the end of the training program, and after confirming their diabetes, treatment was performed. Rats were divided into 5 groups of 5 rats as follows. Additionally, the sample size (n=5 per group) was selected based on previous experimental studies evaluating gene expression changes in diabetic rodent models (13,14): diabetes control (DC), healthy control (HC), quercetin control (QC), HIIT (HIIT), and HIIT training with quercetin (QHIIT). All animals were kept under controlled conditions. The average ambient temperature was 22 ± 3°C, and the light-dark cycle was 12:12 hours. The animals were kept with free access to water and special rat food. Quercetin was injected into the rats every day during these 8 weeks, and the same amount of normal saline was injected into the diabetic control group. The quercetin used in this study was purchased from Sigma Company in powder form with a purity of 85% and was used at a dose of 15 mg/kg as a suspension in 0.5% carboxymethyl cellulose (CMC). Table 1 presents regular diet and HFD.

Exercise protocol

The rats' maximal aerobic speed (MAS) was measured as previously described in another study (15). Thus, the MAS test protocol consisted of a training session in which the starting speed was 10 m/min and gradually increased by 3.33 m/min every 60 seconds until it reached 26.7 m/min, and then by 1.7 m/min until the rats could no longer run. At the end of every two weeks, the maximal MAS of each rat was determined. The HIIT protocol consisted of 8 weeks of treadmill training, 5 times per week, for a total of 40 training sessions. Before and after each HIIT session, a 5-minute exercise bout at 40% of MAS was performed. HIIT sessions consisted of 7-10 training bouts at an intensity of 80-95% of MAS, with 60 seconds of active rest between bouts at 45-50% of MAS. Exercise intensity was gradually increased across the intervention period. Control groups were also brought to the running room every day to ensure identical conditions (From the animal center to the laboratory running room), and these rats were placed on the treadmill while it was turned off. All HIIT sessions were supervised by a graduate exercise physiology student (15). Table 2 presents HIIT training protocol.

Real-time PCR procedure

Within 48 hours after completion of the eight-week training and quercetin injection, and following an overnight fast to eliminate the acute effects of exercise, rats were anesthetized by subcutaneous injection of 20-30 mg/kg of 10% ketamine and 2-3 mg/kg of 2% xylazine. The soleus muscle tissue was rapidly excised and, after washing with physiological saline, placed in RNase- and DNase-free microtubes to prevent contamination and snap-frozen in liquid nitrogen for subsequent mRNA purification and Real-time PCR (RT-PCR). RNA extraction was performed according to the manufacturer's instructions (Pars Tous, Iran). Accordingly, the samples were first powdered under sterile conditions using liquid nitrogen in a porcelain mortar and stored at -70°C. The RNA extraction steps were briefly as follows: first, 750 µL of RL solution was added to microtubes containing 50 mg of powdered tissue, and the contents were thoroughly mixed by pipetting with an appropriate sampler. Subsequently, 200 µL of chloroform was added to each sample and pipetted several times. The samples were then placed in a refrigerator at 4 °C or on a cooling rack for 10 minutes. Next, the samples were centrifuged at 12,000 rpm for 15 minutes. After this period, three phases were formed, with RNA located in the colorless upper aqueous phase. The supernatant was carefully aspirated from the lower phase using a sampler and transferred to a new microtube. Then, 400 µL of cold 75% ethanol was added to the samples; the microtubes were gently inverted and kept at 4 °C for 10 minutes. The samples were transferred to chromatography columns using a sampler and incubated at room temperature for 5 minutes. The columns were centrifuged at 12,000 rpm for 1 minute, and the flow-through was discarded. Then, 700 µL of PW wash buffer was added to the columns, which were centrifuged again at 12,000 rpm for 1 minute, and the flow-through was discarded. Once more, 500 µL of PW wash buffer was added to the columns, followed by centrifugation at 12,000 rpm for 1 minute, and the flow-through was discarded. The columns were then centrifuged empty at 12,000 rpm for 2 minutes, and the flow-through was discarded. After

that, the columns were transferred to new microtubes, and 50 µL of sterile 0.05 M Tris buffer was added to elute the RNA. The columns were then incubated at 55 °C for 5 minutes. The columns were centrifuged at 12,000 rpm for 2 minutes, and the eluted RNA sample was stored in 1.5-mL tubes at -70 °C. To measure mRNA expression, Real-time PCR was performed using a Lava 96 Real-time PCR Detection System (Daan Gene Co. Ltd), and the kit used in the study was 2X SYBR Green Real Time PCR (Pars Tous, Iran). The Real-time PCR reaction was carried out in a total volume containing 6.25 µL of Master Mix, 0.25 µL of forward primer, 0.25 µL of reverse primer, 3 µL of cDNA, and 2.75 µL of water. Relative expression levels of target genes compared with GAPDH expression in each tissue were evaluated using Light Cycler SW1.1 software and reported based on the $2^{-\Delta\Delta Ct}$ method. Real-time PCR reactions on the samples were performed as previously described, with two technical replicates for each sample and each gene, and the mean Ct values of the different dilutions in the two repeats were calculated. For the validity of the method, it is essential that the amplification efficiencies of the target gene and the internal control gene be approximately equal. To this end, a standard curve of the log of the serially diluted standard concentrations of each gene (log input) versus Ct values was plotted. Then, using Excel software, by placing the log of dilution values on the x-axis and the Ct values of each gene on the y-axis, an XY-scatter plot was drawn. The equation of the best-fit line in the form of (y=ax +b) was obtained, and the amplification efficiency of the target and calibrator genes was calculated using the formula (E =10-1/slope). In this formula, E represents amplification efficiency. If the amplification efficiencies of the target and reference genes are similar within a 95% confidence interval (CI=95%), the amplification efficiency of the target genes is considered acceptable. For primer design, gene sequences were retrieved from NCBI GenBank (NUCLEOTIDE section), and primer design was performed using PRIMER3 PLUS software. All primers were designed to span exon-exon junctions, with an optimal melting temperature of 60 °C and a GC content between 45% and 50% (15). Table 3 presents the sequence of the primers used in the present study to investigate MuRF1 and Atrogin-1 gene expression.

Statistical analysis

Data were analyzed using SPSS version 26. Normality of data distribution was evaluated using the Shapiro-Wilk test, and homogeneity of variances was assessed using Levene's test before conducting parametric analyses. One-way ANOVA followed by Tukey's post-hoc test was used to compare differences between groups. Data are presented as mean ± SD. Significance was set at P-Value < 0.05. The overall study design is illustrated in Supplementary Figure 1.

Table 1. Nutritional composition of the standard diet and high-fat diet (HFD) used for induction of obesity and type 2 diabetes in the experimental rats

Diet ingredients	Carbohydrate	Fat	Protein	Fiber	Mineral	Vitamin	Cholesterol powder	Corn oil 100%
Regular diet	70 %	10 %	20 %	50 gr	50 gr	3 gr	-	-
High fat diet	70 %	10 %	20 %	50 gr	50 gr	3 gr	1%	1%

Table 1 shows that both diets were formulated with an identical macronutrient distribution, while the high-fat diet was additionally supplemented with cholesterol powder and corn oil.

Table 2. Eight-week progressive high-intensity interval training (HIIT) protocol, including exercise intensity, training volume, and session duration

Weeks	Intensity (% MAS)	Number of bouts	Bout duration (s)	Active recovery (m/min)	Total exercise time in a session (Min)
1	80	7	60	10	24
2	80	7	60	10	24
3	85	8	60	11	26
4	85	8	60	11	26
5	90	9	60	12	28
6	90	9	60	12	28
7	95	10	60	13	30
8	95	10	60	13	30

Table 2 shows progressive increases in total exercise time, intensity (% MAS), and number of bouts, with constant 60-second work intervals.

Table 3. Primer sequences for quantitative RT-PCR

Genes	MuRF1	Atrogin-1	GAPDH
Forward	ACCTTCCTCTTGAGTGCCAA	TAAGGAGCGCCATGGATACT	AGTTCAACGGCACAGTCAAG
Reverse	TCAAGGCCTCTGCTATGTGT	TCAGCTCCAACAGCCTTACT	TACTCAGCACCAGCATCACC
bp	139	113	119
Accession No.	NM_080903.2	NM_133521.2	NM_017008.4

Table 3 shows primer sequences, amplicon sizes (bp), and NCBI accession numbers for the MuRF1, Atrogin-1, and GAPDH genes used for quantitative real-time PCR (qRT-PCR) analysis in the present study.

Results

Blood glucose

As the variances were equal, one-way ANOVA was used. The results showed a significant difference between groups in blood glucose (P-Value <0.001). Before HFD exposure and STZ injection, all groups (HC, DC, QC, HIIT, and QHIIT) had similar blood glucose values: 97.74 ± 2.93, 94.91 ± 5.50, 93.09 ± 4.89, 94.10 ± 6.8, and 92.15 ± 6.29, respectively. After HFD exposure and STZ injection, blood glucose levels significantly increased in the DC, QC, HIIT, and QHIIT groups compared with the HC group (P-Value <0.001). However, 48 hours after the last training session, a significant reduction was observed in the QC, HIIT, and QHIIT groups compared with the DC group (P-Value <0.001). The blood glucose value remained consistent in the HC group (P-Value =0.10) (Figure 1).

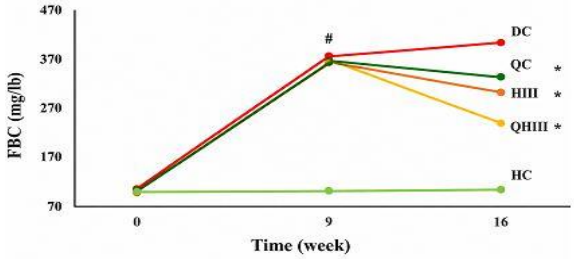

Figure 1. Blood glucose values before STZ injection, one week after STZ injection, and 48 hours after the last intervention session. # Indicates a significant difference in the DC, QC, HIIT, and QHIIT groups compared with the HC group (P-Value <0.001). * Indicates a significant decrease in the QC, HIIT, and QHIIT groups compared with the DC group (P-Value <0.001). Abbreviations: HC: Healthy Control; DC: Diabetic Control; QC: Quercetin Control; HIIT: High-Intensity Interval Training; QHIIT: Quercetin with HIIT.

Gene expression changes

Based on the results of the one-way analysis of variance test, a significant difference was observed between the groups in the gene expression of MuRF1 (P-Value<0.001) and Atrogin-1 (P-Value <0.001). The results of Tukey's post-hoc test for MuRF1 and Atrogin-1 gene expression showed a significant increase in MuRF1 (P-Value =0.001) and Atrogin-1 (P-Value =0.001) in the DC group compared with the HC group, as shown in Figure 2 (A and B).

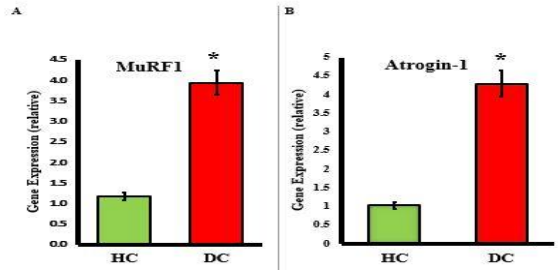

Figure 2. Changes in MuRF1 and Atrogin-1 gene expression in the HC and DC groups. * Indicates a significant increase in the DC group compared with the HC group (P-Value =0.001). Abbreviations: HC: Healthy Control; DC: Diabetic Control.

The results of Tukey's post-hoc test for MuRF1 showed no significant decrease in the QC and HIIT groups compared with the DC group, with significance levels of (P-Value =0.839) and (P-Value =0.371), respectively. A significant decrease was observed only in the QHIIT group compared with the DC group for MuRF1 gene expression, with a significance level of (P-Value =0.001), as shown in Figure 3.

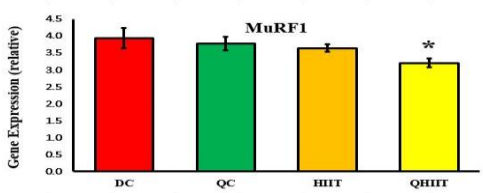

Figure 3. Changes in MuRF1 gene expression in the DC, QC, HIIT, and QHIIT groups. * Indicates a significant decrease in the QHIIT group compared with the DC group (P-Value =0.001). Abbreviations: DC: Diabetic Control; QC: Quercetin Control; HIIT: High-Intensity Interval Training; QHIIT: Quercetin with HIIT.

The results of Tukey's post-hoc test for Atrogin-1 showed no significant decrease in the QC and HIIT groups compared with the DC group, with significance levels of (P-Value =0.104) and (P-Value =0.095), respectively. A significant decrease was observed only in the QHIIT group compared with the DC group for Atrogin-1 gene expression, with a significance level of (P-Value <0.001), as shown in Figure 4.

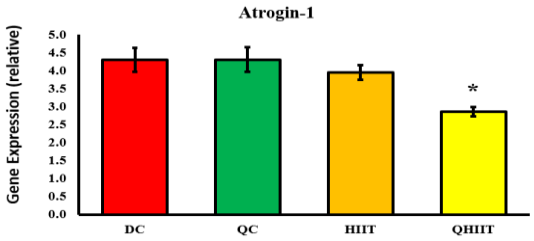

Figure 4. Changes in Atrogin-1 gene expression in the DC, QC, HIIT, and QHIIT groups. * Indicates a significant decrease in the QHIIT group compared with the DC group (P-Value <0.001). Abbreviations: DC: Diabetic Control; QC: Quercetin Control; HIIT: High-Intensity Interval Training; QHIIT: Quercetin with HIIT.

Discussion

In the present study, changes in MuRF1 and Atrogin-1 gene expression following 8 weeks of HIIT combined with quercetin supplementation in the soleus muscle of diabetic rats were investigated. The results of the current study showed that blood glucose significantly decreased after 8 weeks of HIIT, quercetin supplementation, and HIIT with quercetin supplementation. A significant decrease in the expression of both genes was observed only in the HIIT with quercetin supplementation group. Regarding quercetin, it can be stated that quercetin is one of the most important flavonoids and has significant radical-scavenging and antioxidant properties due to its polyphenolic chemical structure (16,17). Polyphenols are a group of biologically active compounds in plants, and flavonoids are among the most important of them, with antiviral, anti-atherosclerotic, cardioprotective, anti-diabetic, antioxidant, and anti-inflammatory properties (16,17). In line with this finding, another study also observed that intervention with quercetin in rats fed a high-fat, high-carbohydrate diet significantly reduced blood glucose (18). In another study in diabetic rats, 30 days of intervention with quercetin significantly reduced plasma glucose and significantly increased glycogen content in muscle and liver tissue as well as hexokinase enzyme activity, indicating improved insulin sensitivity (19). In general, quercetin polyphenols reduce insulin resistance by increasing the transfer of GLUT4 to the muscle and fat cell membranes, along with the induction of AMPK and PI3K pathways (20). Research has confirmed that quercetin increases insulin release and normalizes blood glucose by promoting beta-cell regeneration in pancreatic islets (21).

Although HIIT and quercetin supplementation alone reduced the expression of the MuRF1 and Atrogin-1 genes, this reduction was not significant, and only eight weeks of HIIT with quercetin supplementation reduced MuRF1 and Atrogin-1 gene expression levels. There are some consistent (22) and inconsistent studies (23,24). Moradi et al. (2020) conducted an experimental study to investigate the effects of 8 weeks of endurance, resistance, and concurrent training on Atrogin-1 and MuRF1 gene expression in the vastus lateralis muscle of male Wistar rats. In this study, 35 two-month-old male rats were randomly assigned to endurance, resistance, concurrent training, and control groups. After completion of the training protocol, muscle samples were collected 48 hours later, and gene expression levels were analyzed using the RT-PCR method. The findings demonstrated that endurance training significantly increased MuRF1 and Atrogin-1 gene expression compared with the control group, whereas resistance training significantly decreased the expression of these genes, and concurrent training produced no significant changes compared with the control group. The researchers concluded that resistance training may prevent muscle atrophy by reducing the expression of genes associated with muscle protein degradation, while endurance training may exacerbate muscle atrophy through increased expression of these atrophy-related genes, and concurrent training does not appear to exert a substantial effect on this pathway. The inconsistency with the results of the present study may be attributed to differences in the type of exercise, age,

gender, duration of the intervention, exercise intensity, and nutritional strategies implemented. As observed, in the study by Moradi et al. (2020) (22), the rats were healthy, whereas in the present study, the samples were diabetic. A study has shown that HIIT can alter the expression of Atrogin-1, miR-23a, miR-206, and myostatin genes as effective factors in reducing diabetes complications, including muscle atrophy, due to improvement in hyperglycemia (23). Research suggests that in diabetes, increased levels of MuRF1 and subsequently Atrogin-1 may contribute to muscle degradation. Specifically, diabetes is associated with increased oxidative stress and inflammation, both of which can activate the MuRF1 pathway. Regular exercise has been shown to reduce muscle wasting in neurodegenerative diseases such as T2DM. In this regard, in animal models, exercise can reduce MuRF1 expression and thus protect against muscle atrophy (25). Taken together, the activity of the FoxO3a/MuRF1 pathway, but not the NF-κB/MuRF1 pathway, can promote atrophy due to detraining, and MuRF1 is not always a reliable marker of atrophy (26). This is likely achieved through mechanisms such as activation of the mTOR (Mechanistic target of rapamycin) pathway, which is involved in muscle protein synthesis, and inhibition of protein degradation pathways, including MuRF1 (19). Human studies have shown that resistance and aerobic exercise reduce UPS activity and MuRF1 expression in muscle tissues, which subsequently affects and reduces Atrogin-1. In the study by Zatparvar et al. (2023), the effects of alternating cycles of ischemia with resistance and endurance training for six weeks on the expression of MuRF1 and Atrogin-1 genes and the diameter of gastrocnemius muscle fibers in diabetic rats were investigated. The results indicated a decrease in MuRF1 and Atrogin-1 gene expression and an increase in gastrocnemius muscle diameter in all training groups, and this effect was greater in the groups that underwent ischemic cycles (27). Also, in the study by Madadhi et al. (2022), the effect of reduced physical activity by spinal nerve ligation (SNL) after a period of resistance, endurance, and combined training was evaluated on MuRF1 gene expression in male rats. Finally, they stated that the rats in the resistance training group appeared to be more resistant to atrophy caused by SNL than those in the endurance training group. In addition, the results of this study showed a decrease in MuRF1 gene expression in the combined training/reduced physical activity and resistance training/reduced physical activity groups (28). In the study by Delfan et al. (2021), the effect of an eight-week aerobic training period on MuRF1 and Atrogin-1 values in the soleus muscle of diabetic rats was also investigated. The results indicated a decrease in the levels of the two indices, MuRF1 and Atrogin-1, after a period of aerobic training (24). In the study by Moradi et al. (2020), the effect of an eight-week resistance-training period on MuRF1 and Atrogin-1 in the vastus lateralis muscle of rats was investigated. The results indicated a decrease in the levels of the two indices, MuRF1 and Atrogin-1, after a period of resistance training (22), which is inconsistent with the results of the present study. It is believed that there may be further contradictions regarding the intracellular signaling pathway of the effect of endurance training on atrophy indices, with possible reasons including the research sample, intensity, duration, and volume of training, as well as the type of tissue and its harvesting time. As observed, in the study by Madadhi et al. (2022) (28), healthy samples and resistance training were examined, whereas in the study by Delfan et al. (2021) (24), diabetic samples were studied. Considering that exercise combined with supplementation caused a significant change in the two above-mentioned indicators, it can be stated that both interventions, in a complementary manner, caused changes in genes involved in the atrophy pathway, as quercetin supplementation has been shown to increase anabolic hormones and muscle mass, improve mitochondrial biogenesis, and ameliorate muscle oxidative stress. On the one hand, continuous exercise is thought to inhibit the ubiquitin-proteasome system by suppressing the FOXO and NF-κB signaling pathways, as studies have shown that FOXO binds to DNA sequences in the promoter region of Atrogin-1 and Benip3 and, when activated, causes muscle atrophy along with other myogenic factors (29). On the other hand, TNF-α is thought to increase MuRF1 expression in muscles, which is modulated by continuous exercise through the AMPK pathway, resulting in decreased MuRF1 levels; consequently, the decrease in MuRF1 levels subsequently reduces Atrogin-1 gene expression (30). MuRF1 has been reported to be involved in the loss of muscle mass and function caused by activation of NF-κB and TNF-α (10). The limitations of the present study included the sample size, measurement of mRNA rather than protein, use of one sex and one muscle type, and focus on one dose of supplementation and exercise.

Conclusion

The findings of the present study demonstrated that combined HIIT and quercetin supplementation significantly reduced MuRF1 and Atrogin-1 gene expression in the soleus muscle of obese diabetic rats, whereas HIIT or quercetin supplementation alone did not produce significant molecular alterations. These results suggest that the combined intervention may exert synergistic protective effects against skeletal muscle atrophy associated with T2DM.

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Not applicable.

Ethical Statement

The Ethics Committee of the University of Mohaghegh Ardabili approved this study protocol (IR.UMA.REC.1404.042).

Conflicts of Interest

The equipment necessary for conducting the study was provided by the University of Mohaghegh Ardabili. All authors were involved in data interpretation and presentation and approved the final manuscript.

Author Contributions

A. R and M.Kh played pivotal roles in data collection, statistical population collection, and laboratory coordination. A. R and M.M played pivotal roles in establishing the research background, and their expertise and insights were crucial to the success of this research.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Use of Artificial Intelligence

The authors declare that artificial intelligence tools were used for language editing and improving the clarity of the manuscript. The study design, data analysis, interpretation of results, and final conclusions were performed entirely by the authors, who take full responsibility for the content of this article.

Abbreviations

Abbreviations: T2DM: Type 2 Diabetes Mellitus; DM: Diabetes Mellitus; HIIT: High-Intensity Interval Training; HFD: High-Fat Diet; STZ: Streptozotocin; HC: Healthy Control; DC: Diabetic Control; QC: Quercetin Control; QHIIT: Quercetin with High-Intensity Interval Training; MuRF1: Muscle RING-Finger Protein-1; Atrogin-1: Muscle Atrophy F-box Protein (MAFbx); RT-PCR: Real-Time Polymerase Chain Reaction; qRT-PCR: Quantitative Real-Time Polymerase Chain Reaction; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase; MAS: Maximal Aerobic Speed; ANOVA: Analysis of Variance; SPSS: Statistical Package for the Social Sciences; CMC: Carboxymethyl Cellulose; RNA: Ribonucleic Acid; cDNA: Complementary DNA; NCBI: National Center for Biotechnology Information; Ct: Cycle Threshold; FOXO3a: Forkhead Box O3a; NF-κB: Nuclear Factor Kappa B; TNF-α: Tumor Necrosis Factor Alpha; AMPK: Adenosine Monophosphate-Activated Protein Kinase; mTOR: Mechanistic Target of Rapamycin; IGF-1: Insulin-like Growth Factor-1; GLUT4: Glucose Transporter Type 4.

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