DOI: 10.22122/jrrs.2023.41895.1059 Published by Vesnu Publications

Modulation of Muscle Atrophy in Elderly Prediabetic and Diabetic Rats through Interval Aerobic Exercise and Crocetin Extract: An Experimental Study

Parviz Ehsani-Kolor[1], Khosro Jalali-Dehkordi[2], Farzaneh Taghian[2], Mehdi Kargarfad[3], Seyed Ali Hoseyni[4]

Original Article

Abstract

Introduction: MuRF1 and Foxo3 genes are important molecular markers of muscle atrophy and are significantly increased in skeletal muscle under various conditions, such as diabetes. The aim of the present study was to evaluate the modulation of muscle atrophy in elderly prediabetic and diabetic rats through interval aerobic training and crocetin extract.

Materials and Methods: In this experimental study, 45 elderly male and female c57bl6 mice with diabetes [40 mg/kg peritoneal injection of streptozotocin (STZ)] aged 14-16 weeks and weighing 30-35 grams were randomly divided into 9 healthy control, pre-diabetic, pre-diabetic + aerobic exercise, pre-diabetic + crocetin, pre-diabetic + aerobic exercise + crocetin, diabetic, diabetic + aerobic exercise, diabetic + crocetin, and diabetic + aerobic exercise + crocetin groups. Interval aerobic training was performed for eight weeks, five sessions per week. Mice received crocetin 30 mg/kg/day by peritoneal injection. MuRF1 and Foxo3 expression levels were measured by the real-time polymerase chain reaction (PCR). To analyze the data, one-way analysis of variance (ANOVA) and Tukey's post hoc test were used ($P \leq 0.05$).

Results: MuRF1 and Foxo3 gene expression in the skeletal muscle of the diabetes group was significantly higher than that of the control group ($P = 0.01$). The gene expression levels of MuRF1 and Foxo3 in the pre-diabetes group + aerobic exercise + crocetin supplement and the diabetes group + aerobic exercise + crocetin supplement were significantly lower than those of other groups ($P = 0.01$). Insulin and glucose levels were significantly lower in the pre-diabetic and diabetic groups receiving aerobic exercise and a crocetin supplement than in other groups ($P = 0.01$).

Conclusion: It seems that interval aerobic training and crocetin, alone or synergistically, can help reduce atrophy in elderly people with prediabetes and diabetes by downregulating MuRF1 and Foxo3 in skeletal muscles.

Keywords: Aerobic exercise; Crocetin; Atrophy; Heart; Elderly; Pre-diabetes; Diabetes

Citation: Ehsani-Kolor P, Jalali-Dehkordi K, Taghian F, Kargarfad M, Hoseyni SA. **Modulation of Muscle Atrophy in Elderly Prediabetic and Diabetic Rats through Interval Aerobic Exercise and Crocetin Extract: An Experimental Study.** J Res Rehabil Sci 2022; 18.

Received date: 02.03.2022 Accept date: 02.06.2022 Published: 06.03.2023

Introduction

Diabetes is a metabolic disorder characterized by impaired glucose metabolism, which, in turn, affects lipid and protein metabolism (1). Various studies have shown that diabetes is associated with an increased risk of skeletal muscle atrophy (2-4). Muscle atrophy in chronic conditions such as type 2 diabetes is primarily driven by hyperglycemia and hypoinsulinemia (5). Prolonged muscle atrophy disrupts intracellular signaling pathways that maintain the balance between protein synthesis and degradation (6). Proteolytic pathways, including the ubiquitin-proteasome system, lysosome-mediated degradation, autophagy, and Caspase-3, are responsible for protein breakdown in muscle and contribute to muscle wasting (7). In healthy muscle, the degradation of damaged proteins is essential for maintaining cellular homeostasis; however, in atrophic conditions such as inactivity or

1- PhD Student, School of Physical Education and Sport Sciences, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran
2- Associate Professor, School of Physical Education and Sport Sciences, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran
3- Professor, Department of Physiology, School of Sports Sciences, University of Isfahan, Isfahan, Iran
4- Professor, Department of Exercise Physiology, Marodasht Branch, Islamic Azad University, Marodasht, Iran
Corresponding Author: Khosro Jalali-Dehkordi; Associate Professor, School of Physical Education and Sport Sciences, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran; Email: khosrojalali@khuisf.ac.ir

diabetes, increased activity of these pathways leads to excessive degradation of contractile proteins, resulting in muscle atrophy (8).

Several factors, collectively referred to as atrogenes, contribute to muscle atrophy. MuRF1 (Muscle RING-finger protein-1) is a key atrogene within the ubiquitin-proteasome system, activated by FoxO (forkhead box) transcription factors (9). Activation of MuRF1 may represent an early maladaptive event in certain atrophic conditions. Studies have shown that diabetes increases MuRF1 mRNA expression in diabetic mice (10). Skeletal muscle is composed of multinucleated cells, and improper nuclear positioning is a significant determinant in the pathogenesis of muscular disorders (11).

Insulin binding to its cell-surface receptor promotes IRS-1 (insulin receptor substrate-1) activation, leading to phosphatidylinositol-3,4,5-trisphosphate (PIP3) production via PI3K (phosphatidylinositol-3-kinase) and subsequent activation of AKT (protein kinase B) at the plasma membrane. When the AKT pathway is inactive, muscle atrophy is triggered through activation of the FoxO transcription factor (12). Inactivation of the IGF-1–PI3K–AKT pathway is associated with upregulation of ubiquitin-proteasome ligases, MAFbx, and MuRF1 (13). AKT directly phosphorylates FoxO in response to growth stimuli, retaining FoxO in the cytosol. Upon removal of growth signals, AKT becomes inactive, leading to FoxO dephosphorylation, nuclear translocation, and activation of genes involved in cell cycle inhibition, metabolism, and apoptosis. Animal models demonstrate that FoxO overactivation reduces muscle mass, likely through increased MAFbx and MuRF1 expression (14). In a study on aged subjects, 12 weeks of aerobic exercise reduced FoxO3 mRNA expression, indicating a regulatory effect on atrophic signaling (15).

In recent years, physical exercise has been recognized as an effective intervention capable of inducing neuromuscular adaptations of varying intensities. High-intensity interval training (HIIT) has been supported for patients with type 2 diabetes, involving alternating bouts of very high-intensity activity and active low-intensity recovery periods, which substantially increase the metabolic demands of muscle and the body (16). However, the precise physiological mechanisms through which HIIT improves skeletal muscle health remain unclear. Muscle atrophy results from a negative balance between protein synthesis and degradation. In catabolic states or inactivity, atrophy can reduce functional capacity and quality of life and increase mortality (16).

Additionally, the use of natural antioxidants alongside exercise may enhance health outcomes. Saffron (Crocus sativus) and its derivatives, such as crocetin, have demonstrated significant bioactive properties, including anti-inflammatory and antioxidant effects, inhibition of lipoprotein oxidation, and benefits in coronary artery disease, hypertension, neurodegenerative disorders, and cancer (17).

Given the increasing prevalence of diabetes, its detrimental health effects, and the potential benefits of antioxidant supplementation and structured exercise, the present study aimed to investigate the effects of eight weeks of high-intensity interval aerobic exercise combined with crocetin supplementation on skeletal muscle atrophy-related factors in prediabetic and aged diabetic mice.

Materials and Methods

Animal Maintenance: In this experimental study, 48 female BALB/c mice, aged 14–16 weeks and weighing 30–35 g, were obtained from the Animal Breeding and Research Center at Royan Institute, Isfahan. Upon transfer to the university's specialized Exercise Physiology Laboratory, the animals were acclimated for 1 week under laboratory conditions. Throughout the study, all animals were maintained under standard conditions, including a 12-hour light/dark cycle, ambient temperature of 20-22 °C, relative humidity of 55%, and ad libitum access to food and water. All experimental procedures were conducted in accordance with the Helsinki Declaration and approved by the Ethics Committee of Islamic Azad University, Khorasgan Branch (Approval Code: IR.IAU.KHUISF.REC.1401.386).

Induction of Diabetes: Prediabetic and diabetic aged C57BL/6 mice were fasted for 12 hours prior to induction. Prediabetic mice received a single intraperitoneal injection of streptozotocin (STZ) at 20 mg/kg dissolved in citrate buffer. In contrast, diabetic mice received a single intraperitoneal injection of STZ at 40 mg/kg in citrate buffer. Four days post-injection, blood glucose levels were measured using a glucometer. Mice with blood glucose levels exceeding 250 mg/dL were classified as diabetic (18).

It is noteworthy that three mice died following STZ administration due to individual sensitivity, leaving a total of 45 mice in the experimental groups. These 45 diabetic mice were randomly assigned to the following groups: prediabetic, prediabetic + aerobic exercise, prediabetic + crocetin, prediabetic + aerobic exercise + crocetin, diabetic, diabetic + aerobic exercise, diabetic + crocetin, and diabetic + aerobic exercise + crocetin. Additionally, five healthy mice were included as a

control group to evaluate the effects of pre-diabetes and diabetes induction (15).

High-Intensity Interval Aerobic Exercise Protocol: The exercise groups underwent a 1-week familiarization period on a motorized treadmill at 7-10 m/min for 10 minutes. Following familiarization, a graded exhaustion test was conducted to determine each mouse's maximal running speed for exercise prescription. Mice were first warmed up for 5 minutes at 5 m/min, after which the speed was increased by 1 m/min every 3 minutes until exhaustion. Exhaustion was defined as the inability of the mouse to continue running on the treadmill or to contact the end of the treadmill three consecutive times within one minute.

During the first week, mice performed the adaptation exercise at 7–10 m/min for 10 minutes. Subsequently, the high-intensity interval training (HIIT) protocol was applied, consisting of alternating treadmill speeds of 7, 10, 13, 10, and 7 m/min in the first week. By the eighth week, the speed progression was 10, 13, 16, 25, 19, 16, 13, and 10 m/min. The treadmill speed gradually increased from 7 to 25 m/min over the 8-week training period. HIIT was performed five sessions per week, with a 5-minute warm-up at 50% of maximal running speed at the beginning and a 5-minute cool-down at the same intensity at the end of each session (Table 1) (19).

Crocetin Supplementation: Crocetin (Product No. 6-881-255) was purchased from Sigma-Aldrich, USA. Mice received a daily dose of 30 mg/kg body weight of crocetin via oral gavage (20).

Tissue Dissection and Sampling: Forty-eight hours after the last exercise session, mice in all experimental groups were anesthetized via intraperitoneal injection of ketamine (50 mg/kg body weight) and xylazine (20 mg/kg body weight). After confirming complete anesthesia, defined as the absence of response to applied stimuli, approximately 5 mg of the soleus muscle was excised under sterile conditions. The tissue was then washed with physiological saline and transferred into 1.8 mL microtubes containing RNAlater™ solution (20% v/v), appropriately labeled with the mouse ID and the dissection time. Samples were immediately stored in liquid nitrogen for subsequent analyses.

Gene Expression Analysis of MURF1 and FOXO3: To assess the expression levels of MURF1 and FOXO3, quantitative real-time PCR (qReal-Time PCR) was performed (1). For this purpose, 50 mg of muscle tissue was excised from each animal, and RNA was extracted from all experimental groups according to the manufacturer's protocol (Qiagen, Germany). The quality of the extracted RNA was evaluated using agarose gel electrophoresis and by measuring optical absorbance at 260 nm with a NanoDrop spectrophotometer (Sigma, USA). Additionally, RNA concentration was calculated using the following formula: $C\ (\mu g/\mu L) = (A260 \times \varepsilon \times d) \div 1000$

Subsequently, cDNA synthesis was performed using the Fermentas kit (K1621) following the manufacturer's instructions. Specific primers (Table 2) were designed based on the gene sequences of MURF1 and FOXO3 available on PubMed (https://pubmed.ncbi.nlm.nih.gov). Primer efficiency and specificity were evaluated using software available on NCBI (https://www.ncbi.nlm.nih.gov). TBP was used as an internal control gene to normalize expression levels. After completion of the qReal-Time PCR reaction and reaching the cycle threshold (Ct) for each sample, the relative expression of target genes compared to the reference gene was calculated using the 2^-ΔΔCT method.

Measurement of Serum Insulin and Glucose: Serum glucose concentration was measured using a glucometer (Alpha TRAK, Zoetis, USA), and serum insulin levels were determined using an ELISA kit (Abcam, ab277390, USA).

To ensure normality of the variables, the Shapiro-Wilk test was applied, and homogeneity of variances was assessed using Levene's test. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to analyze the data. A significance level of $P \leq 0.05$ was considered for all analyses. All statistical analyses were performed using SPSS software, version 26 (IBM Corporation, Armonk, NY, USA).

Table 1. High-intensity interval training protocol

Week	Speed (m/min)	Duration (min)	Sessions per week
Adaptation	7–10	10	5
Week 1	7, 10, 13, 10, 7	10, 3, 19, 3, 10	5
Week 2	7, 10, 13, 15, 12, 10, 7	5, 3, 3, 3, 23, 3, 3, 5	5
Week 3	10, 13, 16, 17, 16, 13, 10	5, 3, 3, 23, 3, 3, 5	5
Week 4	10, 13, 16, 18, 16, 13, 10	5, 3, 3, 23, 3, 3, 5	5
Week 5	10, 13, 16, 19, 16, 13, 10	5, 3, 3, 23, 3, 3, 5	5
Week 6	10, 13, 16, 20, 16, 13, 10	5, 3, 3, 23, 3, 3, 5	5
Week 7	10, 13, 16, 21, 18, 15, 12, 10	5, 3, 3, 20, 3, 3, 3, 5	5
Week 8	10, 13, 16, 25, 19, 16, 13, 10	5, 3, 3, 20, 3, 3, 3, 5	5

Table 2. Primer Sequences of the Target Genes

Gene	Primer Sequence	Temperature (°C)
B2m	F: ACAGTTCCACCCGCCTCACATT R: TAGAAAGACCAGTCCTTGCTGAAG	60
MURF1	F: TACCAAGCCTGTGGTCATCCTG R: ACGGAAACGACCTCCAGACATG	60
FOXO3	F: CCTACTTCAAGGATAAGGGCGAC R: GCCTTCATTCTGAACGCGCATG	60

Results

The results of a one-way analysis of variance (ANOVA) demonstrated significant differences in glucose (P = 0.01), insulin (P = 0.01), and gene expression levels of MURF1 (P = 0.01) and FOXO3 (P = 0.01) in skeletal muscle tissue from diabetic rats across the experimental groups.

Post hoc analysis using Tukey's test showed that glucose and insulin levels in the pre-diabetic group were significantly higher than those in the healthy control group (P = 0.01). However, in the pre-diabetic + aerobic exercise, pre-diabetic + crocetin, and pre-diabetic + aerobic exercise + crocetin groups, glucose and insulin levels were significantly lower compared to the pre-diabetic group (P = 0.01).

Similarly, glucose and insulin levels in the diabetic group were significantly elevated compared with those in the healthy control group (P = 0.01). Nonetheless, diabetic + aerobic exercise, diabetic + crocetin, and diabetic + aerobic exercise + crocetin groups showed a significant reduction in glucose and insulin levels compared to the diabetic group (P = 0.01) (Tables 3 and 4).

The Tukey post hoc test indicated that MURF1 levels in the prediabetic groups (P = 0.01) were significantly higher than those in the healthy group. However, in the prediabetic+ aerobic exercise (P = 0.01), prediabetic+ herbal supplement (P = 0.01), and prediabetic+ aerobic exercise + herbal supplement (P = 0.01) groups, MURF1 levels significantly decreased compared to the prediabetic group. Additionally, MURF1 levels in the diabetic groups (P = 0.01) were significantly higher than in the healthy group, but significantly decreased in the diabetic+ aerobic exercise (P = 0.01), diabetic+ herbal supplement (P = 0.01), and diabetic+ aerobic exercise + herbal supplement (P = 0.01) groups compared to the diabetic group.

Similarly, the Tukey post hoc test showed that FOXO3 levels in the prediabetic groups (P = 0.01) were significantly higher than in the healthy group. However, in the prediabetic + aerobic exercise (P = 0.01), prediabetic + herbal supplement (P = 0.01), and prediabetic + aerobic exercise + herbal supplement (P = 0.01) groups, FOXO3 levels decreased significantly compared to the prediabetic group. Furthermore, MURF1 levels in the diabetic groups (P = 0.01) were significantly higher than in the healthy group, but significantly decreased in the diabetic+ aerobic exercise (P = 0.01), diabetic+ herbal supplement (P = 0.01), and diabetic+ aerobic exercise + herbal supplement (P = 0.01) groups compared to the diabetic group.

Discussion

The present study demonstrated that streptozotocin-induced diabetes is associated with skeletal muscle atrophy and increased MuRF1 expression. Additionally, the results showed that 8 weeks of interval training, with or without crocetin supplementation, could reduce MuRF1 expression and mitigate muscle atrophy in diabetic mice.

Table 3. Mean of serum glucose and insulin in experimental groups

Group	Insulin (µIU/mL) (mean ± SD)	P-value	Glucose (mg/dL) (mean ± SD)	P-value
Healthy Control	0.59 ± 0.09	< 0.01	95.4 ± 4.1	< 0.01
Pre-diabetic	1.70 ± 0.02	< 0.01*	142.2 ± 2.5	< 0.01*
Pre-diabetic + Aerobic Exercise	1.20 ± 0.04	< 0.01*	117.2 ± 3.5	< 0.01*
Pre-diabetic + Herbal Supplement	1.14 ± 0.04	< 0.01*	111.0 ± 1.4	< 0.01*
Pre-diabetic + Aerobic Exercise + Supplement	0.84 ± 0.25	< 0.01*	98.5 ± 0.7	< 0.01*
Diabetic	5.20 ± 0.12	< 0.01*	310.2 ± 10.0	< 0.01*
Diabetic + Aerobic Exercise	4.10 ± 0.05	< 0.01*	290.0 ± 0.2	< 0.01*
Diabetic + Herbal Supplement	3.80 ± 0.04	< 0.01*	281.5 ± 2.1	< 0.01*
Diabetic + Aerobic Exercise + Supplement	2.80 ± 0.11	< 0.01*	246.6 ± 1.6	< 0.01*

*Indicates significant difference from the control group at P < 0.05.
SD: Standard deviation

Table 4. Mean of FOXO3 and MURF1 expression in experimental groups

Group	FOXO3 expression (mean ± SD)	P-value	MURF1 expression (mean ± SD)	P-value
Healthy Control	0.99 ± 0.02	< 0.01	0.98 ± 0.02	< 0.01
Pre-diabetic	7.40 ± 0.30	< 0.01*	5.40 ± 0.30	< 0.01*
Pre-diabetic + Aerobic Exercise	6.20 ± 0.21	< 0.01*	4.20 ± 0.09	< 0.01*
Pre-diabetic + Herbal Supplement	5.60 ± 0.12	< 0.01*	3.60 ± 0.10	< 0.01*
Pre-diabetic + Aerobic Exercise + Supplement	4.07 ± 0.52	< 0.01*	2.90 ± 0.04	< 0.01*
Diabetic	11.40 ± 0.25	< 0.01*	12.41 ± 0.11	< 0.01*
Diabetic + Aerobic Exercise	5.40 ± 0.15	< 0.01*	9.40 ± 0.07	< 0.01*
Diabetic + Herbal Supplement	4.70 ± 0.07	< 0.01*	6.70 ± 0.21	< 0.01*
Diabetic + Aerobic Exercise + Supplement	2.70 ± 0.34	< 0.01*	4.70 ± 0.34	< 0.01*

*Indicates significant difference from the control group at $P < 0.05$.
SD: Standard deviation

These findings are consistent with the results of Panahi et al. (21), Liu et al. (22), Ribeiro et al. (23), and Moradi et al. (24).

Limited studies have examined the effect of exercise on MuRF1 expression, and they have suggested different underlying mechanisms. In this regard, Panahi et al. showed that 4 weeks of resistance training reduced MuRF1 gene expression in diabetic Wistar rats (21). Liu et al. also reported that eight weeks of moderate-intensity aerobic exercise decreased MuRF1 in type 2 diabetic mice (22). Pancreatic damage induced by STZ injection in rodents and the increase in MuRF1 expression in skeletal muscle atrophy suggest a potential role for MuRF1 in diabetes-related muscle atrophy (21).

Several studies have attempted to clarify the mechanisms underlying the increased expression of MuRF1 in diabetic muscle. Its expression is regulated by two transcription factors: Foxo and NF-κB. Evidence shows that increased NF-κB activity is necessary for IKKβ-mediated MuRF1 transcription, with at least 50% of the changes in muscle mass attributed to NF-κB activation (25). Since chronic IKK activation occurs in diabetic patients and NF-κB is strongly activated under diabetic conditions, the NF-κB pathway appears to be a major contributor to diabetes-induced MuRF1 upregulation. The transcription factor Foxo is also involved in muscle degradation and likely regulates MuRF1 ubiquitin ligases (26). Inflammation increases MAPKp38 activity via NF-κB, which may drive atrogene expression and protein degradation (atrophy) (27).

Additionally, studies on rodent cells using pharmacological approaches or gene inhibition have shown that MuRF1 transcription is regulated through the AKT-Foxo signaling pathway (9, 28). MuRF1 response to exercise may vary depending on intensity, duration, and repetitive activity. Hsp25 (Heat Shock Protein-25) seems to act as a regulatory mediator of the atrophy pathway, playing a key role in counteracting diabetic muscle atrophy. Increased Hsp25 expression following exercise reduces atrophy and MuRF1 expression, suggesting that exercise may be an effective intervention for diabetes-related muscle atrophy.

Diabetes leads to alterations in cellular proteins, oxidative stress, and impaired cellular defense mechanisms (29). Exercise-induced reduction of oxidative stress in diabetic subjects in this study likely contributed to the downregulation of MuRF1 by the end of the training period. Chen et al. linked increased MuRF1 expression in diabetes-induced atrophy to oxidative stress, showing that H_2O_2 induces MuRF1 expression and MHC degradation in cultured cells (30). Ribeiro et al. also reported that 12 weeks of resistance training decreased MuRF1 in the muscles of young and aged mice (23). Persistent hyperglycemia in diabetes disrupts the pro-oxidant–antioxidant balance, increasing ROS and decreasing antioxidant levels. Insulin reduction and oxidative stress are essential for protein breakdown and muscle atrophy (23, 29).

The current study also showed that Foxo3 expression in skeletal muscle was significantly increased in the diabetic group compared with the other groups. In contrast, exercise, with or without crocetin, significantly attenuated Foxo3 expression. Foxo3 was used as a marker of atrophy and protein degradation. Seven days of immobilization reportedly increased Foxo3 by 82%, MAFbx by 78%, and MuRF1 by 91% (31), consistent with findings from Kang and Li Li Ji (32) and other studies on inactivity and muscle mass (33). Batista et al. (34) demonstrated that denervation increased Foxo3 and MuRF1 expression in muscle fibers, leading to atrophy. In animal models, Foxo3 overexpression caused increased MAFbx and MuRF1 expression, reducing muscle volume, suggesting that these genes are reliable indicators of decreased muscle mass (31).

Multiple mechanisms contribute to muscle atrophy under disuse conditions. IGF-1, through the

AKT-mTOR pathway, plays a key role in skeletal muscle protein synthesis, so its reduction is a significant factor in decreased protein synthesis and subsequent muscle mass loss. AKT inactivation activates Foxo (35). In rodents, increased Foxo reduces insulin receptor substrate availability, and inactivation of the IGF-PI3K-AKT pathway correlates with elevated levels of the E3 ubiquitin ligases MAFbx and MuRF1 (36). In the present study, interval training reduced Foxo3 in aged diabetic mice. AKT directly phosphorylates Foxo in response to growth stimuli, retaining it in the cytosol; upon removal of these stimuli, AKT is inactivated, leading to Foxo dephosphorylation, nuclear translocation, and activation of genes involved in cell cycle regulation, metabolism, and apoptosis (32-34).

Crocetin, with its potent antioxidant and anti-inflammatory properties, reduces oxidative stress and inflammation, inhibits atrophy, and improves muscle health. Combined crocetin supplementation and regular interval exercise protect muscle from oxidative stress by reducing lipid peroxidation and enhancing antioxidant defenses, thereby modulating skeletal muscle atrophy in aged diabetic mice (37).

Limitations

The present study was limited by the lack of measurement of other factors associated with skeletal muscle atrophy and reliance on Western blot techniques.

Recommendations

Future studies should investigate the effects of these interventions on other tissues such as the heart, brain, and liver. Given that interval training and crocetin supplementation modulated muscle atrophy in aged prediabetic and diabetic mice, it is recommended to use aerobic exercise and crocetin, both individually and in combination, to improve muscle atrophy.

Conclusion

The present study demonstrated that 8 weeks of crocetin supplementation and interval aerobic training reduced skeletal muscle atrophy in prediabetic and diabetic mice. Combined interval training and crocetin produced the most significant reduction in muscle atrophy. Therefore, interval aerobic exercise and crocetin supplementation, acting through distinct pathways, effectively modulate skeletal muscle atrophy. Overall, crocetin, with its antioxidant properties, in combination with interval aerobic training, reduces muscle atrophy in prediabetic and diabetic mice.

Acknowledgments

The present study is taken from the doctoral dissertation of Physical Education and Sports Science-Sports Physiology with ethics code IR.IAU.KHUISF.REC.1401386 approved by the Islamic Azad University of Isfahan (Khorasgan).

Authors' Contribution

Project design and ideation: Parviz Ehsani-Kolor, Khosro Jalali-Dehkordi, Farzaneh Taghian, Mehdi Kargarfard, Seyed Ali Hosseini

Providing financial resources for the project: Parviz Ehsani-Kolor, Khosro Jalali-Dehkordi

Scientific and Executive support of the project: Parviz Ehsani-Kolor, Khosro Jalali-Dehkordi, Farzaneh Taghian, Mehdi Kargarfard, Seyed Ali Hosseini

Providing equipment and study samples: Parviz Ehsani-Kolor, Khosro Jalali-Dehkordi, Farzaneh Taghian,

Data collection: Parviz Ehsani-Kolor, Khosro Jalali-Dehkordi, Farzaneh Taghian

Analysis and Interpretation of Results: Parviz Ehsani-Kolor, Khosro Jalali-Dehkordi

Specialized statistics services: Parviz Ehsani-Kolor, Khosro Jalali-Dehkordi, Farzaneh Taghian, Mehdi Kargarfard

Manuscript Peroration: Parviz Ehsani-Kolor, Khosro Jalali-Dehkordi, Farzaneh Taghian, Mehdi Kargarfard, Seyed Ali Hosseini

Specialized Scientific evaluation of the Manuscript: Parviz Ehsani-Kolor, Khosro Jalali-Dehkordi, Farzaneh Taghian, Mehdi Kargarfard, Seyed Ali Hosseini

Confirm of the final manuscript to be submitted to the journal: Parviz Ehsani-Kolor, Khosro Jalali-Dehkordi, Farzaneh Taghian, Mehdi Kargarfard, Seyed Ali Hosseini

Maintaining the integrity of the study process from the beginning to the publication and responding to the reviewers comment: Parviz Ehsani-Kolor, Khosro Jalali-Dehkordi, Farzaneh Taghian, Mehdi Kargarfard, Seyed Ali Hosseini

Funding

The present study is taken from the doctoral dissertation of Physical Education and Sports Science-Sports Physiology with ethics code IR.IAU.KHUISF.REC.1401386 approved by the Islamic Azad University of Isfahan (Khorasgan). The study was conducted without financial support.

Conflict of Interest

The authors have no conflict of interest.

References

1. Rahmati M, Taherabadi SJ. The effects of exercise training on Kinesin and GAP-43 expression in skeletal muscle fibers of STZ-induced diabetic rats. Scientific reports. 2021; 11(1): 9535.
2. Zolfaghari M, Faramarzi M, Hedayati M, Ghaffari M. The effect of resistance and endurance training with ursolic acid on atrophy-related biomarkers in muscle tissue of diabetic male rats induced by streptozotocin and a high-fat diet. Journal of Food Biochemistry. 2022; 46(8): e14202.
3. Catinelli BB, Rossignoli PS, Floriano JF, Carr AM, de Oliveira RG, Dos Santos NJ et al. Reversal of diabetic-induced myopathy by swimming exercise in pregnant rats: a translational intervention study. Scientific Reports. 2022; 12(1): 7375.
4. Akagawa M, Miyakoshi N, Kasukawa Y, Ono Y, Yuasa Y, Nagahata I, Sato C, Tsuchie H, Nagasawa H, Hongo M, Shimada Y. Effects of activated vitamin D, alfacalcidol, and low-intensity aerobic exercise on osteopenia and muscle atrophy in type 2 diabetes mellitus model rats. PLoS One. 2018; 13(10): e020485
5. Ebert SM, Al-Zougbi A, Bodine SC, Adams CM. Skeletal muscle atrophy: discovery of mechanisms and potential therapies. Physiology. 2019; 34(4): 232-9.
6. Lee JH, Jeon JH, Lee MJ. Docosahexaenoic acid, a potential treatment for sarcopenia, modulates the ubiquitin–proteasome and the autophagy–lysosome systems. Nutrients. 2020; 12(9): 2597.
7. Powers SK, Goldstein E, Schrager M, Ji LL. Exercise training and skeletal muscle antioxidant enzymes: An update. Antioxidants. 2022; 12(1): 39.
8. Singh A, Yadav A, Phogat J, Dabur R. Dynamics and Interplay between Autophagy and Ubiquitin-proteasome system Coordination in Skeletal Muscle Atrophy. Current Molecular Pharmacology. 2022; 15(3): 475-86.
9. Madahi M, Gharakhanlou R, Kazemi A, Azarbayjani MA. Effect of reduced physical activity on Murf-1 and Atrogin-1 gene expression in soleus muscle of wistar rats following endurance, resistance and combined training. The Scientific Journal of Rehabilitation Medicine. 2022; 11(2): 250-63.
10. Perry BD, Caldow MK, Brennan-Speranza TC, Sbaraglia M, Jerums G, Garnham, A, et al. Muscle atrophyin patients with Type 2 diabetes mellitus: roles of inflammatory pathways, physical activity and exercise. Exercise Immunology Review. 2016; 22, 94-109.
11. Schulman VK, Folker ES, Rosen JN, Baylies MK. Syd/JIP3 and JNK signaling are required for myonuclear positioning and muscle function. PLoS genetics. 2014 Dec 18; 10(12): e1004880.
12. Ebert SM, Al-Zougbi A, Bodine SC, Adams CM. Skeletal muscle atrophy: discovery of mechanisms and potential therapies. Physiology. 2019; 34(4): 232-9.
13. Bodine SC, Baehr LM. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. American Journal of Physiology-Endocrinology and Metabolism. 2014; 307(6): E469-E84
14. Zeng Z, Liang J, Wu L, Zhang H, Lv J, Chen N. Exercise-induced autophagy suppresses sarcopenia through Akt/mTOR and Akt/FoxO3a signal pathways and AMPK-mediated mitochondrial quality control. Frontiers in Physiology. 2020; 11: 583478
15. Catinelli BB, Rossignoli PS, Floriano JF, Carr AM, de Oliveira RG, Dos Santos NJ, Úbeda LC, Spadella MA, Hallur RL, Sobrevia L, Felisbino SL. Reversal of diabetic-induced myopathy by swimming exercise in pregnant rats: a translational intervention study. Scientific Reports. 2022; 12(1): 7375.
16. Way KL, Sabag A, Sultana RN, Baker MK, Keating SE, Lanting S, Gerofi J, Chuter VH, Caterson ID, Twigg SM, Johnson NA. The effect of low-volume high-intensity interval training on cardiovascular health outcomes in type 2 diabetes: A randomised controlled trial. International journal of cardiology. 2020; 320: 148-54.
17. Su X, Yuan C, Wang L, Chen R, Li X, Zhang Y, et al. The beneficial effects of saffron extract on potential oxidative stress in cardiovascular diseases. Oxidative medicine and cellular longevity. 2021; 19: 1-14.
18. Hasanzadeh Dolatabadi F, Jalali Dehkordi KH, Taghian F, Hoseini SA. The effect of eight weeks of aerobic training with Propolis on some mitochondrial biogenesis markers in cardiac tissue of ovariectomized diabetic rats. Armaghane Danesh. 2022; 27(2): 155-69
19. Haghparast Azad M, Niktab I, Dastjerdi S, Abedpoor N, Rahimi G, Safaeinejad Z, et al. The combination of endurance exercise and SGTC (Salvia–Ginseng–Trigonella–Cinnamon) ameliorate mitochondrial markers' overexpression with sufficient ATP production in the skeletal muscle of mice fed AGEs-rich high-fat diet. Nutrition & metabolism. 2022; 19(1): 17.
20. Zhang J, Wang Y, Dong X, Liu J. Crocetin attenuates inflammation and amyloid-β accumulation in APPsw transgenic mice. Immunity & Ageing. 2018; 15(1): 1-8.
21. Panahi S, Agha-Alinejad H, Gharakhanloo R, Fayazmilani R, Hedayati M, Safarzadeh A, Zarkesh M. The effect of 4 weeks resistance training on murf1 gene expression and muscle atrophy in diabetic wistar rats. Medical Journal of Tabriz University of Medical Sciences. 2016 Jun 12; 38(2): 6-13. [In Persian].
22. Liu HW, Chang SJ. Moderate exercise suppresses NF-κB signaling and activates the SIRT1-AMPK-PGC1α Axis to attenuate muscle loss in diabetic db/db mice. Frontiers in physiology. 2018; 9: 636.
23. Ribeiro MBT, Guzzoni V, Hord JM, Lopes GN, Marqueti RC, Andrade RV, et al. Resistance training regulates gene expression of molecules associated with intramyocellular lipids, glucose signaling and fiber size in old rats. Scientific Report. 2017; 7(1): 8593
24. Moradi Y, Zehsaz F, Nourazar MA. Concurrent exercise training and Murf-l and Atrogin-1 gene expression in the vastus lateralis muscle of male Wistar rats. Apunts Sports Medicine. 2020 Jan 1; 55(205): 21-7.

25. Zhang J, Zheng J, Chen H, Li X, Ye C, Zhang F, Zhang Z, Yao Q, Guo Y. Curcumin targeting NF-κB/ubiquitin-proteasome-system axis ameliorates muscle atrophy in triple-negative breast cancer cachexia mice. Mediators of Inflammation. 2022; 2022(1): 2567150.
26. Li Y, Zhang F, Modrak S, Little A, Zhang H. Chronic alcohol consumption enhances skeletal muscle wasting in mice bearing cachectic cancers: the role of TNFα/myostatin axis. Alcoholism: Clinical and Experimental Research. 2020; 44(1): 66-77.
27. Odeh M, Tamir-Livne Y, Haas T, Bengal E. P38α MAPK coordinates the activities of several metabolic pathways that together induce atrophy of denervated muscles. The FEBS Journal. 2020; 287(1): 73-93.
28. Musi CA, Agrò G, Santarella F, Iervasi E, Borsello T. JNK3 as therapeutic target and biomarker in neurodegenerative and neurodevelopmental brain diseases. Cells. 2020; 28; 9(10): 2190.
29. Shabani M, Valipour-Dehnou V, Tabandeh MR, Molanouri-Shamsi M. The effect of aerobic endurance exercise on changes in heat shock protein 60 and insulin resistance in mice with type 2 diabetes. Feyz Medical Sciences Journal. 2022; 26(3): 273-81.
30. Chen GQ, Mou CY, Yang YQ, Wang S, Zhao ZW. Exercise training has beneficial anti-atrophy effects by inhibiting oxidative stress-induced MuRF1 upregulation in rats with diabetes. Life Sciences. 2011; 89(1-2): 44-9.
31. Wong S, Bhasin S, Serra C, Yu Y, Deng L, Guo W. Lopinavir/Ritonavir impairs physical strength in association with reduced Igf1 expression in skeletal muscle of older mice. Journal of AIDS & clinical research. 2013; 4: 216
32. He W, Wang P, Chen Q, Li C. Exercise enhances mitochondrial fission and mitophagy to improve myopathy following critical limb ischemia in elderly mice via the PGC1a/FNDC5/irisin pathway. Skeletal muscle. 2020; 10(1): 25.
33. Sandri M. Protein breakdown in cancer cachexia. In Seminars in cell & developmental biology; 2016 (54, pp. 11-19). Academic Press.
34. Baptista IL, Leal ML, Artioli GG, Aoki MS, Fiamoncini J, Turri AO, et al. Leucine attenuates skeletal muscle wasting via inhibition of ubiquitin ligases. Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine. 2010; 41(6): 800-8
35. Reddy SS, Shruthi K, Joy D, Reddy GB. 4-PBA prevents diabetic muscle atrophy in rats by modulating ER stress response and ubiquitin-proteasome system. Chemico-biological interactions. 2019; 306: 70-7.
36. Islam H, Hood DA, Gurd BJ. Looking beyond PGC-1α: emerging regulators of exercise-induced skeletal muscle mitochondrial biogenesis and their activation by dietary compounds. Applied Physiology, Nutrition, and Metabolism. 2020; 45(1): 11-23.
37. Cerdá-Bernad D, Valero-Cases E, Pastor JJ, Frutos MJ. Saffron bioactives crocin, crocetin and safranal: Effect on oxidative stress and mechanisms of action. Critical Reviews in Food Science and Nutrition. 2022; 62(12): 3232-49.