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Periodic Aerobic Exercise and the Active Compound Crocetin Causing Improvement in Autophagy Signaling Pathways in Cardiac Tissue of Aged Pre-Diabetic and Diabetic Mice: An Experimental Study

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Original Article

Abstract

Introduction: Type 2 diabetes is characterized by elevated serum glucose and impaired insulin function. Exercise and crocetin supplement are known as two effective factors in preventing the complications of type 2 diabetes. The present study investigated the effects of aerobic exercise and the effective compound crocetin on the autophagy signaling pathway in a heart tissue experimental model of elderly pre-diabetic and diabetic mice.

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. Autophagy-related gene 5 (ATG5) and light chain 1 (LC1) expression levels were measured by 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: The expression of ATG5 in the pre-diabetes group + aerobic exercise + crocetin supplement and the diabetes group + aerobic exercise + crocetin supplement was significantly higher than that in other groups ($P = 0.01$). LC1 gene expression in the pre-diabetes group + aerobic exercise + crocetin supplement and the diabetes group + aerobic exercise + crocetin supplement was significantly lower than that in other groups ($P = 0.01$). Moreover, the insulin and glucose levels in the pre-diabetes group + aerobic exercise + crocetin supplement and the diabetes group + aerobic exercise + crocetin supplement were significantly lower than those in the other groups ($P = 0.01$).

Conclusion: It seems that interval aerobics and crocetin are effective, alone and synergistically, in improving autophagy in heart tissue in pre-diabetic and diabetic mice. Therefore, the use of periodic aerobic exercise and crocetin is recommended in senile pre-diabetes and diabetes.

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

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Introduction

Diabetic cardiomyopathy (DCM) is a distinct pathological condition associated with cardiac dysfunction. This disorder leads to cardiomyocyte loss and contributes to cardiovascular diseases such as atherosclerosis and hypertension. However, the precise molecular mechanisms underlying cell death in DCM remain unclear (1).

Autophagy is a conserved cellular process in which double-membrane vesicles sequester damaged proteins and organelles and deliver them to lysosomes for degradation and energy production (2). It acts as a

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catabolic mechanism that maintains cellular energy homeostasis and recycles cytoplasmic components, including unused macromolecules and invading pathogens. One of the key signaling pathways involved in cardiac autophagy is the LC3-I/ATG5 pathway (3). Diabetes has been shown to induce autophagy in cardiomyocytes through this signaling mechanism.

A pivotal protein in this process is autophagy-related gene 5 (ATG5) (4), which participates in the nucleation of the phagophore — a cup-shaped membrane precursor that gives rise to the pre-autophagosomal structure (PAS). The PAS contains several autophagy-related membrane proteins, including Beclin-1 (5). ATG5 forms a complex with ATG12 and ATG16, catalyzing the conjugation of LC3-I (light chain 3-I) to phosphatidylethanolamine, resulting in the formation of LC3-II on the autophagosomal membrane. Evidence suggests that ATG5 plays a critical role in several human diseases, including cardiomyopathy (6). Altered expression of ATG5 has been observed in neurodegenerative disorders and type 2 diabetes, and ATG5 polymorphisms have been linked to coronary artery disease, acute myocardial infarction, autoimmune disorders (such as lupus), and various cancers (7).

In animal models, cardiac overexpression of ATG5 activates autophagy, enhances insulin sensitivity, and extends lifespan. In contrast, cardiac-specific ATG5 suppression induces cardiomyopathy characterized by mitochondrial collapse, disrupted sarcomeric structure, left ventricular dilation, impaired cardiac function, and premature death (8).

Autophagy involves the formation of double-membrane vesicles that engulf portions of cytoplasm and organelles, which subsequently fuse with lysosomes to degrade their contents. Approximately 30 autophagy-related genes regulate this process. Among them, LC1 plays a crucial role in mammalian autophagy. It is commonly used as a marker for autophagosome formation, with the number of observed autophagosomes serving as an indicator of autophagic activity (9).

MAP1B-LC1 (microtubule-associated protein 1B light chain 1) is centrally involved in regulating Syntaxin 17 (Stx17) function. MAP1B-LC1 links Stx17 to microtubules, preventing its association with ATG14L and autophagosome formation in nutrient-rich conditions. The MAP1B polyprotein is expressed not only in neurons but also in non-neuronal cells (10).

Jafari et al. reported that high-intensity interval training, with or without caffeine administration, modulated the expression of autophagy-related proteins in diabetic mice. Their findings suggested that interval training could serve as a preventive strategy to mitigate diabetes-induced myocardial autophagy (11).

Physical activity, a practical non-pharmacological approach to cardiovascular disease, reduces cardiac risk factors, prevents myocardial damage, and enhances cardiac performance. In recent years, exercise training has been recognized as a potent physiological intervention capable of eliciting cardiovascular adaptations and conferring metabolic, anti-inflammatory, and cardioprotective benefits. Consequently, many researchers have proposed exercise as a significant therapeutic tool for preventing and managing cardiovascular disorders. Furthermore, combining exercise with natural antioxidants may produce more favorable effects on autophagy and cardiac health (12).

Among natural antioxidants, saffron (Crocus sativus L.) and its derivatives have demonstrated remarkable pharmacological and biological activities. Crocetin, one of saffron's key bioactive compounds, exhibits potent anti-inflammatory and antioxidant properties through the inhibition of lipoprotein oxidation, and it has been reported to exert protective effects against coronary artery disease, hypertension, neurodegenerative disorders, and cancer (13). Crocetin has also shown cardioprotective effects in norepinephrine-induced cardiac hypertrophy by inhibiting lipid peroxidation and enhancing antioxidant enzyme activities, including catalase, superoxide dismutase, and glutathione peroxidase (14).

Given the growing prevalence of diabetes and its detrimental impact on cardiovascular health, along with the potential synergistic benefits of antioxidant supplementation and physical exercise, and considering the limited studies on the effects of aerobic interval training combined with crocetin on cardiac autophagy in pre-diabetic and aged diabetic mice, the present study aimed to investigate the effects of eight weeks of aerobic interval training combined with crocetin administration on autophagy-related factors in cardiac tissue of pre-diabetic and aged diabetic mice.

Materials and Methods

Animal Care: In this experimental study, 48 male C57BL/6 mice, aged 14-16 weeks and weighing 30-35 g, were obtained from the Royan Institute's Laboratory Animal Breeding and Reproduction Center (Isfahan, Iran). After transfer to the specialized laboratory, the animals were housed for one week to acclimate to the new environment. Throughout the entire experimental period, the mice were maintained under standard laboratory conditions, including a 12-hour light/dark cycle, ambient temperature of 20-22

°C, relative humidity of 55%, and free access to food and water (11). All ethical principles for working with laboratory animals were strictly followed in accordance with the Helsinki Declaration, and the study was approved by the Ethics Committee of Islamic Azad University, Isfahan (Khorasgan) Branch, under the ethical code IR.IAU.KHUISF.REC.1401.390.

Induction of Diabetes: Elderly pre-diabetic C57BL/6 mice, after 12 hours of fasting, received a single intraperitoneal injection of streptozotocin (STZ; 20 mg/kg) dissolved in citrate buffer. Similarly, elderly diabetic C57BL/6 mice, after 12 hours of fasting, received a single intraperitoneal injection of STZ (40 mg/kg) dissolved in citrate buffer. Four days after the STZ injection, blood glucose levels of the mice were measured using a glucometer. In this study, mice with blood glucose levels higher than 250 mg/dL were considered diabetic (15).

It should be noted that three mice died after the induction of diabetes due to individual sensitivity to STZ, and finally, 45 mice were included in the experimental groups. The 45 diabetic mice were randomly divided into the following groups: 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. It should also be mentioned that to evaluate the effects of pre-diabetic and diabetic induction, five healthy mice were included in the healthy control group (6, 11).

Aerobic Interval Training Protocol: After 1 week of familiarization with the treadmill at 7-10 m/min for 10 minutes, the training groups performed an exhaustive running test to determine the maximum running speed for exercise design. To obtain the maximum running speed of the mice, they first performed a 5-minute warm-up at 5 m/min, then increased the speed by 1 m/min every 3 minutes until exhaustion. Exhaustion was defined as the point at which the mouse was no longer able to continue running on the treadmill or when it touched the end of the treadmill belt three consecutive times within one minute. During the first week, the adaptation phase of training was performed at 7–10 m/min for 10 minutes. Thereafter, aerobic interval training was conducted, consisting of alternating running speeds of 7, 10, 13, 10, and 7 m/min in the first week, and speeds of 10, 13, 16, 25, 19, 16, 13, and 10 m/min in the eighth week. The running speed during the aerobic interval sessions was progressively increased from 7 to 25 m/min over the 8-week training period. Aerobic interval training was performed for eight weeks, five sessions per week. Each session included a 5-minute warm-up and a 5-minute cool-down. The intensity during warm-up and cool-down periods corresponded to 50% of the maximum running speed (Table 1) (16).

Administration of Crocetin: Crocetin supplement (Product No. 6-881-255) was purchased from Sigma-Aldrich (USA) and administered daily at a dose of 30 mg/kg body weight to each mouse via oral gavage (17).

Dissection and Sample Collection: 48 hours after the last exercise session, and following a 12-hour fast, the rats were anesthetized with a solution of ketamine (50 mg/mL) and xylazine (20 mg/mL). The depth of anesthesia was confirmed by pain reflex tests performed by laboratory specialists. After ensuring complete anesthesia, the thoracic cavity was opened, and non-cardiac tissues were carefully displaced. The inflow and outflow arteries of the heart were ligated, and the cardiac tissue was carefully excised. The harvested heart tissue was immediately immersed in liquid nitrogen and subsequently stored at −80°C until further analysis.

Measurement of LC1 and ATG5 Gene Expression: The expression levels of LC1 and ATG5 were measured using quantitative real-time PCR (qRT-PCR) (4). Briefly, 50 mg of cardiac tissue was collected from each sample, and total RNA was extracted from all experimental groups according to the manufacturer's protocol (Qiagen, Germany). RNA quality was assessed by agarose gel electrophoresis and spectrophotometric measurement at 260 nm using a PicoDrop device (Sigma, USA). Additionally, RNA concentration was calculated using the formula:

(C(\mu g/\mu L) = A-{260}\times\varepsilon \times d/1000).

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	60
	R: TAGAAAGACCAGTCCTTGCTGAAG	
Lc1	F: ATTGAGAAGTTGAAAGGAATCCATG	53
	R: GCCCAGTTCGTTTCAGTGCCA	
ATG5	F: CTTGCATCAAGTTCAGCTCTTCC	58
	R: AAGTGAGCCTCAACCGCATCCT	

Complementary DNA (cDNA) was synthesized using the Fermentas kit (K1621) according to the manufacturer's instructions, and primers designed based on the LC1 and ATG5 gene sequences obtained from PubMed (https://pubmed.ncbi.nlm.nih.gov) were used for reverse transcription. Primer efficiency and specificity were evaluated using available NCBI software (https://www.ncbi.nlm.nih.gov).

For gene expression quantification, the housekeeping gene TBP was used as an internal control. Following completion of the qRT-PCR and attainment of cycle threshold (Ct) values, relative gene expression was calculated using the 2 - ΔΔCT method (Table 2).

Measurement of Serum Insulin and Glucose: Serum glucose concentration was measured using a glucometer (AlphaTRAK, Zoetis, USA), and serum insulin levels (ab277390) were determined using an ELISA kit from Abcam according to the manufacturer's instructions.

Data were first tested for normality using the Shapiro-Wilk test. Since the data were normally distributed, one-way analysis of variance (ANOVA) was performed to evaluate differences between groups. Tukey's post hoc test was used to determine the specific group differences. All statistical analyses were conducted using SPSS software, version 26 (IBM Corporation, Armonk, NY, USA).

Results

One-way analysis of variance (ANOVA) revealed significant differences in glucose (P = 0.01), insulin (P = 0.01), and cardiac tissue expression of ATG5 (P = 0.01) and LC1 (P = 0.01) among diabetic rat groups. Post hoc analysis using Tukey's test showed that pre-diabetic rats had significantly higher glucose and insulin levels compared to the healthy control group (P = 0.05). However, pre-diabetic rats subjected to aerobic exercise (P = 0.05), herbal supplementation (P = 0.05), or a combination of aerobic exercise and herbal supplementation (P = 0.05) showed a significant reduction in glucose and insulin levels compared to the untreated pre-diabetic group. Similarly, diabetic rats exhibited significantly higher glucose and insulin levels compared to healthy controls (P = 0.05). Nevertheless, diabetic rats in the aerobic exercise (P = 0.05), herbal supplementation (P = 0.05), and combined aerobic exercise plus herbal supplementation groups (P = 0.05) showed a significant decrease in these parameters compared to the untreated diabetic group (Table 3).

Post hoc Tukey's test revealed that ATG5 expression was significantly lower in the pre-diabetic group than in the healthy control group (P = 0.01). However, pre-diabetic rats subjected to aerobic exercise (P = 0.01), herbal supplementation (P = 0.01), or a combination of aerobic exercise and herbal supplementation (P = 0.01) showed a significant increase in ATG5 expression compared to the untreated pre-diabetic group. Similarly, ATG5 expression in diabetic rats was significantly lower than in healthy controls (P = 0.01).

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

Group	Insulin (µIU/mL) (mean ± SD)	P-value	Glucose (mg/dL) (mean ± SD)	P-value
Healthy Control	0.59 ± 0.09	< 0.05	95.4 ± 4.1	> 0.05
Pre-diabetic	1.7 ± 0.02	< 0.05*	142.2 ± 2.5	< 0.05*
Pre-diabetic + Aerobic Exercise	1.2 ± 0.04	< 0.05*	117.2 ± 3.5	< 0.05*
Pre-diabetic + Herbal Supplement	1.14 ± 0.04	< 0.05*	111 ± 1.4	< 0.05*
Pre-diabetic + Aerobic Exercise + Supplement	0.84 ± 0.25	< 0.05*	98.5 ± 0.7	< 0.05*
Diabetic	5.2 ± 0.12	< 0.05*	310.2 ± 10	< 0.05*
Diabetic + Aerobic Exercise	4.1 ± 0.054	< 0.05*	290 ± 0.16	< 0.05*
Diabetic + Herbal Supplement	3.8 ± 0.04	< 0.05*	281.5 ± 2.07	< 0.05*
Diabetic + Aerobic Exercise + Supplement	2.8 ± 0.11	< 0.05*	246.6 ± 1.6	< 0.05*

*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	LC1 Expression (mean ± SD)	P-value	ATG5 Expression (mean ± SD)	P-value
Healthy Control	0.98 ± 0.01	< 0.01	0.98 ± 0.02	< 0.01
Pre-diabetic	5.5 ± 0.01	< 0.01*	0.31 ± 0.014	< 0.01*
Pre-diabetic + Aerobic Exercise	3.6 ± 0.015	< 0.01*	0.45 ± 0.005	< 0.01*
Pre-diabetic + Herbal Supplement	2.7 ± 0.007	< 0.01*	0.52 ± 0.01	< 0.01*
Pre-diabetic + Aerobic Exercise + Supplement	2.8 ± 0.007	< 0.01*	0.71 ± 0.014	< 0.01*
Diabetic	8.8 ± 0.07	< 0.01*	0.11 ± 0.01	< 0.01*
Diabetic + Aerobic Exercise	6.2 ± 0.21	< 0.01*	0.32 ± 0.007	< 0.01*
Diabetic + Herbal Supplement	4.38 ± 0.21	< 0.01*	0.4 ± 0.01	< 0.01*
Diabetic + Aerobic Exercise + Supplement	3.7 ± 0.04	< 0.01*	0.61 ± 0.005	< 0.01*

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

However, diabetic rats in the aerobic exercise (P = 0.01), herbal supplementation (P = 0.01), and combined aerobic exercise plus herbal supplementation groups (P = 0.01) exhibited a significant increase compared to untreated diabetic rats (Table 3).

Furthermore, Tukey's post hoc analysis indicated that LC1 expression was significantly elevated in pre-diabetic rats compared to healthy controls (P = 0.01). However, pre-diabetic rats receiving aerobic exercise (P = 0.01), herbal supplementation (P = 0.01), or both interventions combined (P = 0.01) showed a significant reduction in LC1 expression compared to the untreated pre-diabetic group. Similarly, LC1 expression in diabetic rats was significantly higher than in healthy controls (P = 0.01), while diabetic rats subjected to aerobic exercise (P = 0.01), herbal supplementation (P = 0.01), or the combination treatment (P = 0.01) demonstrated a significant decrease compared to untreated diabetic rats (Table 4).

Discussion

The results of this study demonstrated that crocetin supplementation and aerobic exercise significantly reduced insulin and glucose levels and decreased LC1 and ATG5 gene expression in both pre-diabetic and diabetic groups. Notably, the combined intervention of exercise and crocetin supplementation resulted in the most pronounced reductions. Diabetes encompasses a broad spectrum of complications, with cardiovascular disease and diabetic cardiomyopathy (DCM) being among the most critical. Previous studies have shown that positive lifestyle modifications, such as a healthy diet and physical activity, can ameliorate metabolic disturbances associated with diabetes through mechanisms including autophagy (18).

The present study aimed to investigate the effects of 8 weeks of aerobic exercise combined with crocetin supplementation on autophagy signaling pathways in the cardiac tissue of aged pre-diabetic and diabetic mice. Consistent with expectations, STZ-induced diabetes led to elevated serum glucose and insulin levels. After 8 weeks of aerobic exercise and crocetin supplementation, glucose and insulin levels improved compared with the diabetic control group. The increase in glucose and insulin in diabetic mice is likely due to pancreatic β-cell damage. Possible mechanisms for glucose regulation in response to aerobic exercise include increased glucose uptake in skeletal muscles via insulin-dependent and independent pathways, enhanced GLUT-4 transporter expression, and increased glycogen synthase activity (19). Additionally, aerobic exercise has been shown to reduce insulin resistance and improve skeletal muscle glucose metabolism by decreasing fat mass and increasing Akt (Protein Kinase B) phosphorylation (20). Behaein et al. reported that aerobic interval training improved glucose and insulin levels in type 2 diabetic mice (19).

Autophagy is a protective system that maintains cellular homeostasis by recycling damaged organelles and protein aggregates. In the present study, diabetes and pre-diabetes decreased ATG5 expression in the cardiac tissue of aged mice, whereas aerobic exercise and crocetin supplementation increased ATG5 levels, which play a crucial role in cardiovascular protection. ATG5 forms a complex with ATG7, contributing to LC3 lipidation and autophagosome membrane maturation (21). Therefore, upregulation of ATG5 indicates enhanced autophagic activity. Increased expression of these genes may reflect enhanced autophagy in cardiac tissue of pre-diabetic and type 2 diabetic individuals. Zhang et al. reported reduced levels of LC3-II, ATG5, and ATG7 in diabetic groups compared to controls (22). As previously noted, absence or impairment of autophagy poses significant challenges, and proper induction of autophagy may reduce cardiomyocyte death and cardiomyopathy (23). Several studies have explored the role of autophagy in

diabetic heart failure through genetic manipulation, pharmacological interventions, and lifestyle modifications, including diet and exercise (23, 24).

In the current study, aerobic exercise and crocetin were investigated as modulators of autophagy at the transcriptional level. Our findings indicate that high-intensity interval training (HIIT) exerts protective effects against DCM in diabetic mice, improving the expression of autophagy-related genes compared to diabetic controls. Exercise serves as a protective factor to maintain normal cellular homeostasis. Crocetin, another independent variable examined, significantly increased transcription levels of Beclin-1 and ATG5 compared with diabetic controls. Zhang et al. analyzed podocytes and kidney tissue from diabetic mice treated with curcumin for 8 weeks, showing significant increases in Beclin-1, ATG5, and autophagy in podocytes (25).

Previous studies also report that a lack of ATG5 reduces mitochondrial abundance and oxidative homeostasis in cardiomyocytes under stress. Functional cytoplasmic alterations and nucleoplasmic Ca^{2+} translocation, mediated by increased CaMKII activity and transcriptional regulation, compromise cardiac reserve capacity and precede heart failure in mice and humans (26). In this study, diabetes and pre-diabetes increased LC1 expression in aged mouse cardiac tissue, whereas aerobic exercise and crocetin reduced LC1 levels. Gao et al. demonstrated that diabetes induced by a high-fat diet and STZ injection elevated autophagy markers (LC3-II/LC3-I ratio, Beclin-1), decreased p62, and increased fibrosis (27). Kanamori et al. showed that STZ-induced diabetes reduced diastolic function while increasing autophagic activity (LC3-II/LC3-I, p62, cathepsin D, autophagic vacuoles, lysosomes), suggesting that the PI3K-Akt signaling pathway regulates autophagy in diabetic hearts (2). In contrast, Molz et al. reported reduced autophagy-related gene (p62/SQSTM1, ATG14) and protein (LC3-II, TGtg5) expression in skeletal muscle of patients with type 2 diabetes (28).

The present study also found that continuous exercise intervention in diabetic mice exerted protective effects against excessive autophagy compared to diabetic controls. Frizen et al. reported that acute knee-extension exercise at 80% peak workload for 60 minutes reduced LC3 lipidation by 50%, decreased the LC3-II/LC3-I ratio, and decreased autophagosome content in the vastus lateralis muscle of healthy men (29). Overexpression of MAP1B-LC1 inhibits the formation of LC3-positive structures, and phosphorylation/dephosphorylation regulates MAP1B-LC1 interaction with Stx17. Diabetes induces dissociation of LC1 from Stx17, leading to rapid activation of autophagy (30). The present study demonstrates that aerobic exercise and crocetin supplementation attenuate diabetes-induced myocardial LC1 expression. Jafari et al. reported that high-intensity interval training, with or without caffeine, modulates autophagy-related proteins in diabetic mice, suggesting HIIT as a preventive strategy for diabetic myocardial autophagy (31).

Recent studies indicate that regular physical activity enhances antioxidant capacity, reduces oxidative stress, and likely mitigates apoptosis, thereby alleviating disease severity. Exercise not only promotes mitochondrial biogenesis but also facilitates the removal of damaged mitochondria through mitochondrial dynamics and autophagy (32). Crocetin, with its potent antioxidant and anti-inflammatory properties, reduces oxidative stress and inflammation while inhibiting apoptosis, improving disease outcomes. It appears that combined crocetin supplementation and regular aerobic exercise protect the heart against oxidative stress by reducing lipid peroxidation and activating antioxidant defenses (33).

Limitations

Considering the role of ATG5 and LC1 isoforms in cardiac autophagy and their sensitivity to exercise, a limitation of the present study is the lack of measurement of specific isoforms. Future studies are recommended to employ complementary methods, such as Western blot and ELISA, to provide a more comprehensive assessment of autophagy-related proteins.

Recommendations

To extend the findings of this study, future research should investigate the effects of aerobic exercise and crocetin supplementation on other tissues, such as skeletal muscle, brain, and liver. Given that the current results indicate improvements in cardiac autophagy in pre-diabetic and aged diabetic mice, it is suggested that both interventions—exercise and crocetin supplementation—be considered individually and in combination to optimize autophagy regulation in the heart.

Conclusion

The present study demonstrates that eight weeks of crocetin supplementation and high-intensity interval aerobic exercise increased ATG5 expression and decreased LC1 expression, indicating enhanced cardiac autophagy in pre-diabetic and aged diabetic mice. Moreover, the combined intervention of aerobic exercise and crocetin exhibited the most significant effect on modulating cardiac autophagy. Overall,

crocetin supplementation, with its antioxidant properties, in combination with aerobic interval exercise effectively improves cardiac autophagy in pre-diabetic and diabetic mice.

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Conflict of Interest

The authors have no conflict of interest.

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