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Effect of high-intensity interval training and moderate-intensity continuous training with quercetin supplementation on the mitochondrial gene expression in the diabetic heart

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Abstract

Background: Mitochondrial function is an integral part of glucose-stimulated insulin secretion in pancreatic β-cells and is a hallmark feature of cardiovascular disease. It may contribute to the pathophysiology of diabetic cardiomyopathy and atherosclerosis. This study aimed to investigate the effect of high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT), combined with quercetin supplementation (Eight weeks), on mitochondrial gene expression in the diabetic heart.

Methods: In this study, 35 adult male rats were equally divided into seven groups (n=5): healthy sedentary, diabetic sedentary, diabetic quercetin sedentary, diabetic HIIT (DHIIT), diabetic MICT (DMICT), DHIIT with quercetin, and DMICT with quercetin. The rats were fed a high-fat diet for eight weeks and subsequently treated with a single low dose of streptozotocin to create a model of type 2 diabetes mellitus (T2DM). Eight weeks (Five times a week) of HIIT and MICT, with and without quercetin, were conducted for the training groups, and quercetin was injected over eight weeks at a dose of 15 mg/kg.

Results: Eight weeks of quercetin supplementation, HIIT, and MICT, with and without quercetin, significantly decreased blood glucose levels (P-Value=0.001). Eight weeks of HIIT and MICT training increased nuclear respiratory factor-2 (NRF2) (P-Value =0.001) and adipose triglyceride lipase (ATGL) (P-Value =0.001) expression and decreased perilipin 2 (PLIN2) gene expression (P-Value =0.001).

Conclusion: The training groups alone improved the gene expression of NRF2, ATGL, and PLIN2. Both training protocols, combined with quercetin, controlled blood glucose levels and improved antioxidant capacity. Thus, the reduction in blood glucose through quercetin supplementation appears to be a promising approach for managing T2DM.

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Highlights

What is current knowledge?

It has been shown that mitochondrial dysfunction is the leading cause of metabolic disorders and heart diseases related to the insulin resistance and T2DM. There are not sufficient studies about the effect of different exercise intensities with quercetin supplementation in diabetic condition.

What is new here?

Quercetin supplementation could control blood glucose in diabetic status. Different intensities of exercise could affect gene expression in diabetic heart.

Introduction

Nowadays, a constellation of metabolic abnormalities and disorders, including atherogenic dyslipidemia (Characterized by low plasma high-density lipoprotein-cholesterol and high plasma triglyceride (TG) concentrations), insulin resistance, B-cell dysfunction, some types of cancer, prediabetes, nonalcoholic fatty liver disease, and type 2 diabetes mellitus (T2DM), is induced by the accumulation of excessive adiposity (1). In general, a progressive increase in the risk of developing T2DM is associated with a corresponding rise in body mass index (BMI), which provides an index of body adiposity. The number of people with diabetes is expected to increase by 42% (From 51 to 72 million) in industrializing countries. Over the past decades, obesity and diabetes have become a growing global problem, with diabetes especially becoming a major concern for healthcare systems and societies (2). We propose that the failure of adipose tissue begins when this tissue cannot increase its cellularity due to an excess fat contribution, whether from genetic or environmental factors. Therefore, obesity-related adverse health consequences appear to be more closely associated with fat distribution rather than the total amount of fat (3).

Diabetes is associated with several pathological findings in the heart, including dysregulated metabolism, lipid accumulation, oxidative stress, and inflammation. Emerging evidence suggests that mitochondrial dysfunction may be a central mediator of these pathological responses (4). Mitochondria are the primary source of adenosine triphosphate (ATP), providing the metabolic needs of the heart. In addition, mitochondrial dysfunction has been shown to be the leading cause of metabolic disorders and heart diseases related to insulin resistance and diabetes (5). Therefore, focusing on mitochondria could be an excellent approach to restoring energy balance in heart diseases related to diabetes. An important factor in cardiac mitobiogenesis is nuclear respiratory factor-2 (NRF2), which transcriptionally regulates the mitochondrial electron transport chain subunits encoded by the nuclear genome (6). Specifically, NRF2 is known to positively regulate nuclear respiratory factor-1 (NRF1) by binding to the four ARE promoter sequences of NRF1, thereby leading to the activation of the NRF1-mediated mitochondrial biogenesis pathway (7).

Intracellular accumulation of lipid droplets (LDs) in the heart, known as cardiac steatosis, correlates significantly with diabetes and obesity (8). Access to the LD surface is tightly regulated by perilipins (PLINs), a class of LD-associated proteins that control the storage and release of LDs (9). Among them, perilipin 2 (PLIN2) is known to regulate cardiac lipophagy (10), and other studies have shown that its expression is increased in various cardiomyopathies (11), particularly in diabetic cardiomyopathy (12). Adipose triglyceride lipase (ATGL) is another indicator that affects fat reserves and is recognized as a critical triglyceride lipase (TAG). It is responsible for breaking down fat reserves into free fatty acids and glycerol (13). In fact, ATGL is one of the LD proteins considered an essential factor in intramuscular TG hydrolysis (14).

Increased exercise training and decreased inflammation levels are associated with a reduced risk of T2DM and obesity (15). Regular exercise not only helps prevent T2DM but also improves diabetes-related indicators such as BMI, blood glucose, insulin sensitivity, lipid profile, oxidative stress/antioxidant capacity, and inflammation (16,17). Physical training can improve glucose metabolism; however, the optimal type, volume, intensity, and frequency are not well understood. High-intensity interval training (HIIT), an emerging exercise type implicated as a time-efficient method to improve metabolic health, requires more investigation in comparison to traditional moderate-intensity continuous training (MICT). HIIT has been found to be more effective than MICT in improving T2DM, insulin sensitivity, mitochondrial biogenesis, glucose regulation, HDL cholesterol, blood pressure, cardiorespiratory fitness, and skeletal muscle strength (18). Quercetin is one of the most extensively studied and abundant dietary flavonols, with beneficial effects such as antioxidant activity, lowering blood glucose, dilating blood vessels, anti-inflammatory, anti-apoptotic, anti-atherogenic, and reducing blood lipids (19). Most of these properties are associated with quercetin's well-known antioxidant potential, which varies with the number, type, and position of glycation. Numerous signaling pathways and elements related to the pathophysiology of T2DM appear to be impacted by quercetin (20). Some research findings have reported the positive effects of exercise on mitochondrial gene expression in healthy animals (21-23) However, no research has apparently examined the effect of quercetin supplementation combined with HIIT and MICT training on the gene expression of NRF2, ATGL, and PLIN2 in the diabetic heart of obese rats. Therefore, this study aimed to investigate the effect of HIIT and MICT with quercetin supplementation on mitochondrial gene expression in the diabetic heart of obese rats.

Methods

Experimental animals

Thirty-five Wistar rats (Weight: 125-130 g, age: eight weeks) were purchased from the Animal Care Center of the University of Mohaghegh Ardabili. To induce T2DM, the rats were exposed to a high-fat diet (HFD) for eight weeks, followed by a single low dose of 25 mg/kg i.p. streptozotocin (STZ) (24). Non-diabetic rats (HS group) were injected with an equivalent volume of citrate buffer. To prepare HFD, 1% cholesterol powder and 1% special 100% corn oil were added to standard food (Table 1). The rats were divided into seven groups (n=5) based on a previous study (24): healthy sedentary (HS), diabetic sedentary (DS), diabetic quercetin sedentary (DQS), diabetic HIIT (DHIIT), diabetic MICT (DMICT), DHIIT with quercetin supplementation (DQHIIT), and DMICT with quercetin supplementation (DQMICT). Six groups of rats were subjected to HFD, while the healthy control group received a regular diet with free access to water. Quercetin was injected intraperitoneally into the DQS, DQHIIT, and DQMICT groups daily during eight weeks of exercise training, and the same amount of normal saline was injected into the other groups. The quercetin used in this study was purchased in powder form from Sigma, with a purity of 85%. It was administered at a dose of 15 mg/kg as a suspension in carboxymethyl cellulose at a concentration of 0.5% (25).

MICT and HIIT training protocol

Both the MICT and HIIT protocols consisted of an eight-week treadmill exercise program with a frequency of five times per week, totaling 40 training sessions. The MICT protocol included a 10-minute warm-up at 33-49% of the rat's maximal aerobic speed (MAS), followed by 50 minutes of running at 65% of MAS, and concluded with a 3-minute active recovery at 20-30% of the rat's MAS (26). The highest running speed and duration were recorded to determine the MAS values. At the beginning of the program and after two, four, six, and eight weeks, the MAS values for each rat were assessed. The MAS test protocol involved a training session starting at 10 m/min, progressively increasing every 60 seconds by 3.33 m/min until reaching 26.7 m/min, and then by 1.7 m/min until the rats could no longer continue running (27). HIIT sessions consisted of 7-10 exercise bouts at an intensity between 80-95% of MAS, followed by 60 seconds of active recovery at an intensity of 45-50% of MAS. Before and after each HIIT session, the rats underwent a 5-minute treadmill exercise at 10 m/min for warm-up and cool-down (Figure. 1).

Body weight, blood glucose levels, and assessment of GPX, SOD and CAT activity

Body weight (BW) values were collected three times: before HFD exposure, after HFD exposure, and 48 hours after the last training session. Blood was collected from the tail vein before HFD, one week after STZ injection, and 48 hours after the last training session while in a state of overnight fasting. To assess glutathione peroxidase (GPX), superoxide dismutase (SOD) values, and catalase (CAT), a portion of the heart tissue was centrifuged at 9,000 rpm for 20 minutes, and the obtained supernatant was used to measure GPX, SOD, and CAT activity. The GPX enzyme accelerated the oxidation of glutathione in the presence of cumene hydroperoxide. In the presence of the glutathione reductase enzyme and nicotinamide dinucleotide phosphate, oxidized glutathione was quickly reduced, and NADPH was converted to NADP+. The presence of the GPX enzyme was determined by measuring the color reduction created during the wavelength of 340 nm ultraviolet rays within 2 minutes. Its activity was reported based on the international protein unit IU/mg. The measurement of SOD activity is based on the inhibition of nitrotetrazolium regeneration by the xanthine-xanthine oxidase system as a producer of superoxide. The light absorption of each sample was read every 30 seconds for 5 minutes at a wavelength of 550 nm. To obtain the percentage of inhibition of nitrotetrazolium regeneration by the SOD enzyme, the formula provided in the Kit Randox manual was used. According to the percentage of inhibition on the standard curve, the activity of the SOD enzyme was obtained, and its activity was reported based on the international unit of protein IU/mg. CAT enzyme activity was measured using the Aebi method at 25 degrees Celsius with a spectrophotometer (Based on the analysis of hydrogen peroxide by the spectrophotometer at 240 nm for 3 minutes), and its activity was reported based on the international unit of protein ng/ml.

Heart tissue extraction and real-time PCR

Forty-eight hours after eight weeks of interventions, following a night of fasting, the rats were anesthetized by intraperitoneal injection of 20-30 mg/kg of 10% ketamine and 2-3 mg/kg of 2% xylazine. The heart tissues were then quickly separated, washed with physiological serum, and frozen in liquid nitrogen in microtubes free of RNAse and DNAse to prevent contamination for mRNA purification and real-time PCR. RNA extraction was performed using the Total RNA Extraction kit reagent according to the manufacturer's instructions (Pars Toss, Iran). After extracting RNA, real-time PCR was used to measure the expression of mRNA with the Lava 96 Real-time PCR Detection System (Daan Gene Co Ltd). The kit used in the research was also 2X SYBR Green Real-Time PCR (Pars Toss, Iran). The real-time PCR reaction was performed with 6.25 microliters of Master Mix, 0.25 microliters of forward primer, 0.25 microliters of reverse primer, and three microliters of cDNA, along with 2.75 microliters of water. Comparative expression values of the NRF2, ATGL, and PLIN2 genes were evaluated against the expression of GAPDH in each tissue using Light Cycler SW1.1 software. The results were reported based on the relationship 2-ΔΔCt. The real-time PCR reaction was performed on the samples according to what was previously described, repeating it two times for each sample and each gene, and the average Ct values of different dilutions were calculated in two repetitions. The sequences of the primers are shown in Table 2.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). The Shapiro-Wilk test confirmed the normality of the data, while Levene's test confirmed the homogeneity of all variances. A mixed-model analysis of variance (ANOVA) with repeated measures across time and groups was used to test the main effect and interaction of blood glucose levels, body weight, and MAS values. The Bonferroni post-hoc test was used for pairwise comparisons. One-way ANOVA was used to test the GPX, SOD, and CAT activity, as well as NRF2, ATGL, and PLIN2 gene expression values between different groups after eight weeks of interventions. The Tukey post-hoc test was used to analyze the variance for pair comparisons. All statistical analyses were conducted at a significance level of $P < 0.05$ and were performed using SPSS software (version 26).

Table 1. Regular diet and HFD

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

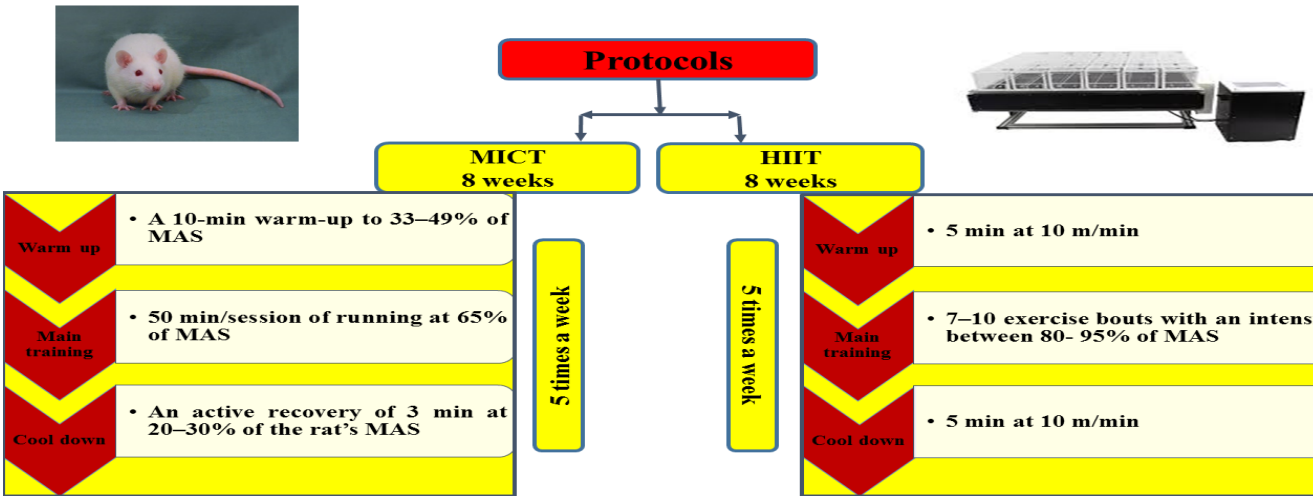

Figure 1. Protocols content

Table 2. The sequence of primers for quantitative real-time PCR

Genes	Forward	Reverse	bp	Accession No.
GAPDH	AGTTCAACGGCACAGTCAAG	TACTCAGCACCAGCATCACC	119	XM_017593963.1
NRF2	ATCCAAGACCAGGGTGGCTG	ACGAGTGCAAGGCAGTGGGA	176	XM_015478844.1
ATGL	TGGCATCAGTAGCAGTGGAT	GGGCATTGGCAACAATCTCA	110	NM_001108509.2
PLIN2	TTTCCAACATGCTGCCAGTG	AGCCACTCCAACAAACGGAT	125	NM_001400556.1

Results

Blood glucose

The results showed a significant difference between the groups for blood glucose (P-Value =0.001). The mixed-model analysis of variance revealed a significant time effect (F2, 56= 1597.48, P-Value <0.001), group effect (F6, 28= 63.47, P-Value <0.001), and an interaction between time and group (F12, 56= 52.922, P-Value <0.001). All groups had similar blood glucose values (P-Value =0.876) before HFD exposure and STZ injection. Blood glucose levels significantly increased in the DS, DHIIT, DMICT, DSQ, DQHIIT, and DQMICT groups compared to the HS group (P-Value <0.001) after HFD exposure and STZ injection. However, a significant reduction was observed in the DHIIT, DMICT, DSQ, DQHIIT, and DQMICT groups compared to the DS group (P-Value <0.001) 48 hours after the last training session. Blood glucose levels remained consistent in the HS group (P-Value >0.1) (Figure 2).

Body weight

Before HFD, all groups had similar body weight values (P-Value =0.328). The results of the mixed-model analysis of variance showed a significant time effect (F2, 56= 6565.83, P-Value <0.001), group effect (F6, 28= 81.22, P-Value <0.001), and an interaction between time and group effect (F12, 56= 67.37, P-Value <0.001) for BW values. Body weight significantly increased in the DS, DHIIT, DMICT, DSQ, DQHIIT, and DQMICT groups compared to the HS group (P-Value =0.001) after eight weeks of HFD. However, a significant reduction was revealed in the DHIIT, DMICT, DQHIIT, and DQMICT groups compared to the DS and DQS groups (P-Value =0.001) 48 hours after the last training session (Figure 3).

SOD, GPX and CAT activity

The result of the one-way ANOVA for SOD, GPX, and CAT activity showed a significant difference between groups. Their values decreased in the DS group compared to the HS group (P-Value <0.05) and increased in the DHIIT, DMICT, DQS, DQHIIT, and DQMICT groups compared to the DS group (P-Value <0.05) (Table 3).

NRF2, PLIN2, and ATGL gene expression

Gene expression results in heart tissues are shown in Figure 4 (A, B, and C) and Figure 5 (A, B, and C). There was a significant decrease in the gene expression levels of NRF2 (Figure 4A) and ATGL (Figure 4B), and a significant increase in the gene expression of PLIN2 (Figure 4C) in the DS group compared to the HS group (P-Value <0.001). The gene expression of NRF2 showed a significant increase in the DHIIT, DMICT, DQHIIT, and DQMICT groups compared to the DS group (P-Value <0.05) (Figure 5A). The gene expression of PLIN2 showed a significant decrease in the DHIIT, DMICT, DQHIIT, and DQMICT groups compared to the DS group (P-Value <0.05) (Figure 5B). The expression of ATGL showed a significant increase in the DHIIT, DMICT, DQHIIT, and DQMICT groups compared to the DS group (P-Value <0.05) (Figure 5C).

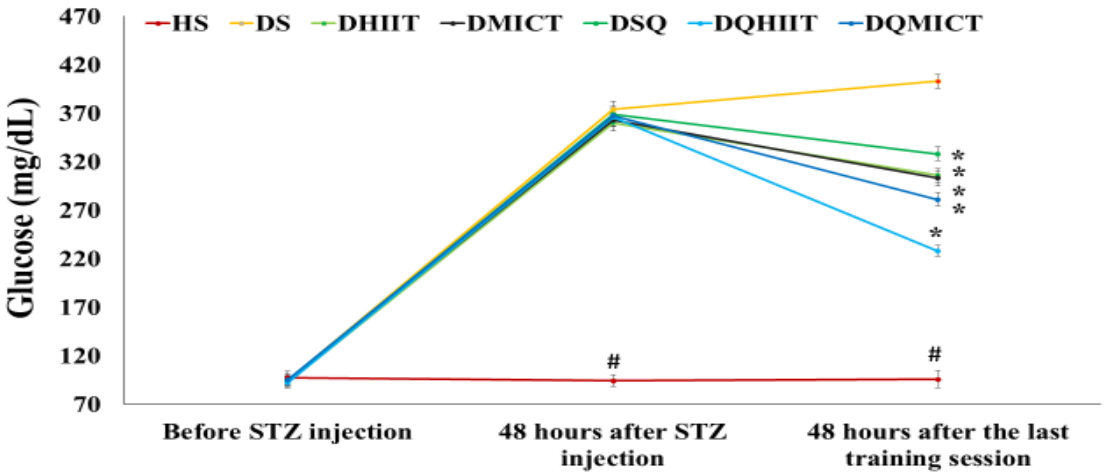

Figure 2. Represents blood glucose values before STZ injection, one week after STZ injection, and 48 hours after the last training session. # indicates a significant difference in the HS group compared to DS, DHIIT, DMICT, DSQ, DQHIIT, and DQMICT groups. * Indicates a significant decrease in DHIIT, DMICT, DSQ, DQHIIT, and DQMICT groups compared to the DS group

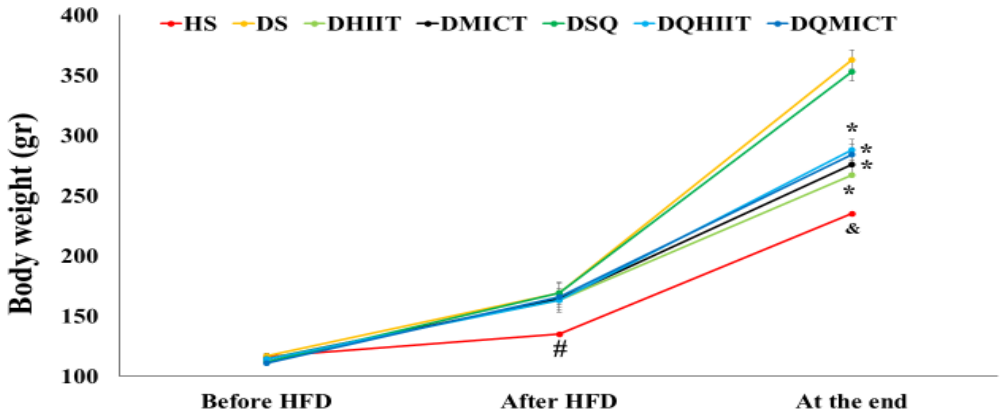

Figure 3. Represents body weight values before starting interventions, after eight weeks of HFD, and 48 hours after the last training session. # indicates a significant difference in the HS group compared to the DS, DHIIT, DMICT, DSQ, DQHIIT, and DQMICT groups before starting the main interventions (Training and quercetin supplementation injection) or after eight weeks of HFD and STZ injection. * Indicates a significant decrease in the DHIIT, DMICT, DQHIIT, and DQMICT groups compared to the DS and DSQ groups, and indicates a significant difference between the HS group compared to the DS, DHIIT, DMICT, DSQ, DQHIIT, and DQMICT groups after eight weeks of main interventions

Table 3. The mean and standard deviation of SOD, GPX and CAT in all groups after eight weeks of interventions

Factors/Groups	HS	DS	DHIIT	DMICT	DSQ	DQHIIT	DQMICT	P-value
SOD (IU/l)	37.50±10.76	27.35±7.57*	39.35±8.86#	35.66±8.96#	33.45±7.54#	36.45± 6.45#	40.33±11.17#	0.001
GPX (IU/l)	145.40±20.17	242.60±13.60*	207.80±14.2#	209.40±21.1#	232.60±12.40#	200.20±20.66#	201.40±13.2#	0.001
CAT (ng/ml)	0.96±0.10	0.49±0.04*	0.71±0.07#	0.69±0.10#	0.65±0.09#	0.78±0.08#	0.81±0.11#	0.001

* Indicates a significant difference between DS group compared to the HS group and # indicates a significant difference between DHIIT, DMICT, DSQ, DQHIIT, and DQMICT groups compared to the DS group

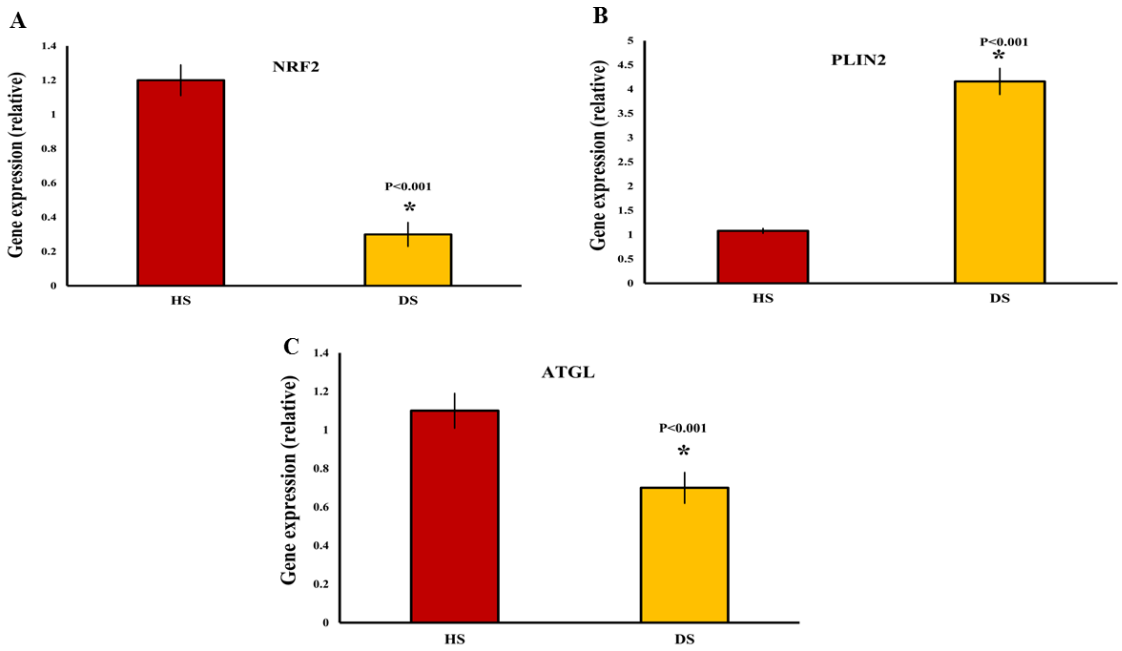

Figure 4. (A, B, and C). Comparison of NRF2 (Figure. 4A), PLIN2 (Figure. 4B), and ATGL (Figure. 4C) gene expression in the DS group compared to the HS group 48 hours after the last training session (Mean ± SD). * Indicates a significant change in the DS group compared to the HS group. Abbreviations: Diabetic Sedentary (DS) and Healthy Sedentary (HS)

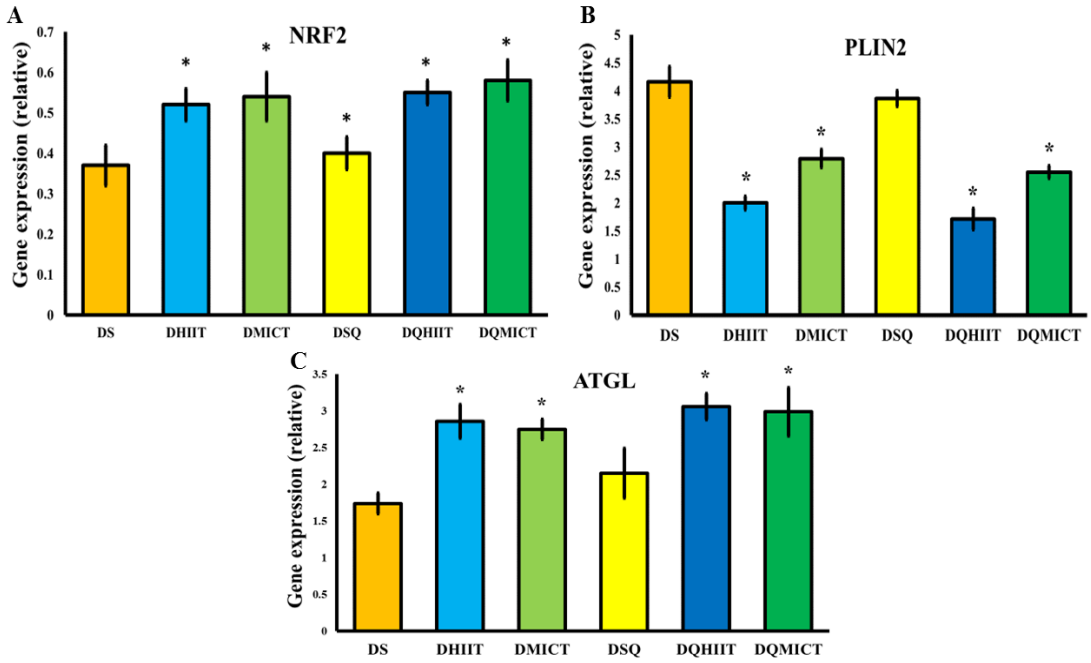

Figure 5. (A, B, and C). Comparison of NRF2 (Figure. 5A), PLIN2 (Figure. 5B), and ATGL (Figure. 5C) gene expression between DS, DHIIT, DMICT, DSQ, DQHIIT, and DQMICT groups 48 hours after the last training session (Mean ± SD). * Indicates a significant increase in NRF2 and ATGL and a significant decrease in PLIN2 in the DHIIT, DMICT, DQHIIT, and DQMICT groups compared to the DS group

Discussion

The present study showed that after inducing T2DM, blood glucose levels significantly increased. Regarding the effect of quercetin injection, HIIT, and MICT on blood glucose levels, results showed that quercetin and both types of exercise alone reduced glucose levels, and the reduction was more noticeable when both exercises were combined with quercetin injection. Quercetin supplementation 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 (28). In this regard, the potential anti-diabetic effects of quercetin supplementation have been observed in human and animal

laboratory studies, involving several basic mechanisms, including stimulation of insulin secretion and antioxidative and anti-inflammatory protection of the pancreas under diabetic conditions (25). Studies show that aerobic exercises increase insulin signaling and glucose transporter content in skeletal muscle. These improvements involve elevated peripheral insulin sensitivity and responsiveness, as well as increased suppression of hepatic glucose production, thereby reducing the risk of T2DM.

As our results revealed, the activity of some enzymatic antioxidants (SOD, GPX, and CAT) in induced T2DM rats significantly decreased compared to the HS group. However, their activity increased after eight weeks of quercetin injection, indicating that GPX, SOD, and CAT were considerably elevated to provide an antioxidant effect, which aligns with the effects of quercetin itself (29,30). Quercetin, a natural polyphenolic flavonoid, has been shown to provide antioxidant properties. It was also reported that quercetin's anti-inflammatory effect is due to the inhibition of proinflammatory cytokines, ATP, and nuclear factor kappa B binding sites (31). On the other hand, physical exercise may play a protective role against oxidative imbalance and heart damage caused by homocysteine induction by increasing the antioxidant activities of enzymes SOD, CAT, and GPX in the rats' heart membranes. In addition, NRF2 can regulate the expression of many antioxidants, such as glutathione reductase (GR), GPX, CAT, and SOD, as demonstrated by the increase in its gene expression through exercise.

Mitochondrial gene expression can be induced by exercise, cold exposure (Thermogenesis), fasting, inflammatory cell stress, and oxidative stress (32). The main finding of the current study was an improvement in mitochondrial gene expression in the diabetic hearts of obese rats. It should be noted that although quercetin supplementation somewhat improved gene expression levels, these changes were not statistically significant, which may be related to the quercetin dosage. NRF2 has been found to have antioxidant and mitochondrial protective properties, making it a target for pharmaceutical interventions in T2DM treatments (33). Studies on NRF2 in humans are limited. According to Done et al. (2016) (34), nuclear content was significantly increased only in young males, not in older males, while the cellular level of NRF2 rose considerably in both groups following a 30-minute cycling test. It has been shown that training can raise the levels of NRF2 in the cells of both young and adult males. However, this does not guarantee nuclear localization or expression of the antioxidant system, most likely due to age-related biological changes. This supports previous conclusions that responses vary according to age, training modality, training duration, and other conditions. In addition, NRF2 influences the functionality of mitochondrial complexes I and II, impacting the production of Kreb's cycle substrates, oxygen consumption, ATP production, and the balance of the NADH redox index, which are involved in mitochondrial function (35). This results in more energy available for physical work during exercise training. NRF2 connects energy, antioxidant, and detoxifying pathways in a complex communication network with biofeedback to each other during activation in training, amplifying its significance.

The other indicators measured in the present study were PLIN2 and ATGL gene expression, which significantly increased and decreased, respectively, after T2DM. However, HIIT and MICT training protocols significantly decreased and increased their values, respectively. Some studies (36-39) are consistent, while others (40,41) are inconsistent with the present study's findings. For example, in the study by Yang et al. (2022), 15 weeks of MICT on the treadmill reduced protein levels of PLIN2 in the livers of rats (41). In Pinode et al.'s study (2019), intense exercise training had different effects on healthy and obese rats. No significant change was observed in the obese rats on an HFD, but its level increased in healthy rats (42), which is inconsistent with the results of the present study. An increase in ATGL levels after exercise has been observed in studies by Fitch and Sugimoto (2021) (38). However, in another study, no changes were observed after endurance exercise (43), and a decrease in ATGL protein levels was seen after 15 weeks of MICT (41). The inconsistency in research findings may be due to factors such as the age and gender of subjects, health status (Healthy or sick), the tissue measured, the laboratory method of analysis, and the type of training protocol used in the study. It has been suggested that there is a relationship between PLIN2 levels and intramuscular triglyceride (IMTG). Although IMTG changes were not investigated in the present study, the training intensity in the HIIT and MICT groups ranged from 65% to 90% of MAS, which, according to other research, is sufficient to alter IMTG levels. Therefore, the significant decrease in PLIN2 levels observed after eight weeks of MICT and HIIT training, with or without quercetin injection, may be due to changes in IMTG levels. Exercise training likely affects PLIN2 and ATGL, reducing fat accumulation and preventing or reducing insulin resistance in subjects with high insulin resistance.

The increase in ATGL gene expression in the MICT group seems expected. Regarding HIIT exercise training, the increase in ATGL gene expression likely helps maintain low concentrations of FA metabolites, ultimately improving insulin sensitivity during intense exercise (44). Recently, Wu et al. (2022) suggested that aerobic exercise is closely related to the reduction of fat accumulation and inflammatory response through the inhibition of the steroid receptor RNA activator (SRA) levels, a long non-coding RNA that has gained attention due to its important role in lipid metabolism (45). Aerobic exercise may inhibit FOXO1 transcriptional activity by suppressing SRA expression, leading to the upregulation of ATGL expression. In addition, in the mentioned study, improvements in inflammation were associated with significant changes in inflammatory proteins and the P38/JNK signaling pathways. Therefore, one of the controlling factors for ATGL gene expression in the diabetic heart may be SRA, as it is a critical lncRNA that potentially improves the inflammatory response through the MAPK signaling pathway (45).

Limitations of the current study include the need for other variables involved in the lipogenesis and prophase measurement process and protein expression of the mentioned indicators. Furthermore, it is essential to study the effects of training volume, frequency, intensity, and modality to tailor specific conditions for each subject at any given time. In addition, since this dose of quercetin did not statistically affect gene expression, the optimal duration and dosage of quercetin supplementation remain unknown. Addressing these limitations in future studies is necessary to confirm the actual effects of quercetin on diabetic conditions.

Conclusion

Both exercises protected mitochondrial biogenesis gene expression, antioxidant capacity, and blood glucose levels in diabetic rats. These changes were more effective when training protocols were combined with quercetin supplementation. While quercetin supplementation did not significantly affect changes in gene expression, it did control blood glucose levels and improve antioxidant capacity.

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Ethical statement

The Ethics Committee of the University of Mohaghegh Ardabili approved the protocol for this study (IR.UMA.REC.1402.042).

Conflicts of interest

All authors contributed to data interpretation and presentation and approved the final manuscript.

Author contributions

Mojdeh Khajehlandi played pivotal roles in data collection, statistical population collection, and laboratory coordination. Mojdeh Khajehlandi and Lotfali Bolboli played crucial roles in establishing the research background, and their expertise and insights were essential to the success of this study.

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