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Experimental Research Article

Impact of two-month sodium citrate supplementation along with moderate-intensity continuous training on PGC-1α and Nrf2 expression in diabetic rats

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ABSTRACT

Introduction: Sports activity increases PGC1α and Nrf2, the regulatory factors of mitochondrial biogenesis. This paper aims to study the impact of two-month sodium citrate supplementation with Moderate-Intensity Continuous Training (MICT) on PGC-1α and Nrf2 expression in diabetic rats.

Methods: Forty-five three-month-old male Wistar rats were haphazardly assigned to one of five equal groups (N=9): (1) healthy; (2) diabetic; (3) diabetes + exercise (DE); (4) diabetes+ supplementation (DS); and (5) diabetic + exercise + supplementation (DSE), matched according to their weights. After induction, exercises began on a treadmill for 8 weeks, five days a week. The MICT protocol ran at 70% of their maximum speed for 36 minutes. The rats supplemented with sodium-citrate- at 15 mmol/L in drinking water for two months. PGC-1α and Nrf2 expression were measured through Western blotting in the soleus muscle. Data were analyzed using univariate analysis of variance (ANOVA) and the Tukey post-hoc test. Cohen's D effect size (ES) was calculated to compare the groups.

Results: The results showed that induction of diabetes significantly reduced the expression of PGC-1α ($P< 0.001$; ES=1.36) and Nrf2 ($P<0.088$; ES=0.24), while exercise increased PGC-1α expression ($P<0.001$; ES=0.68). Sodium citrate supplementation, either alone or in combination with MICT activity, did not show a clear advantage for Nrf2 expression.

Conclusion: MICT activity and sodium citrate supplementation, by increasing PGC-1α expression, can be considered therapeutic strategies for diabetic patients. However, to increase Nrf2 expression, further studies with different exercise intensities and doses of sodium citrate supplementation are needed.

Keywords:
Diabetes mellitus
Exercise
Gene expression
KEAP1 protein
PPARGC1A protein

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Introduction

The continuous increase in diabetes mellitus (DM) worldwide is closely relates to lifestyle changes (Cho et al., 2018). This metabolic disorder disrupts carbohydrates and lipid metabolism, leading to the production of harmful metabolites (Zaccardi et al., 2016). Increased production of reactive oxygen species (ROS) in mitochondria due to oxidative stress induces insulin resistance by affecting the insulin signal transduction pathway directly. It can also indirectly impair insulin signaling by causing mitochondrial damage and mitophagy (Rabah et al., 2023). Mitochondria are the main source of free radicals, contributing to insulin resistance through oxidative stress (Sergi et al., 2019). In contrast, peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α) is an activator that does not directly bind to DNA. Instead, PGC-1α controls the activity of various transcription factors involved in cellular energy metabolism, regulating mitochondrial biogenesis, respiration, gluconeogenesis, glucose transport, glycogenolysis, fatty acid oxidation, peroxisomal recovery, muscle fiber transformation, and oxidative phosphorylation (Corona and Duchen 2015).

Thus, PGC-1**α** is a master regulator of mitochondrial biogenesis (Wu et al., 2016), expressed in various organs including the liver and muscles, with skeletal muscle being the primary site of glucose and fatty acid utilization.

Glucose absorption and utilization are critical limiting factors in skeletal muscle energy metabolism and homeostasis. Consequently, researchers have identified a connection between impaired PGC-1α regulation in skeletal muscle and abnormal energy balance, as well as insulin sensitivity in type 2 diabetes. PGC-1α expression is lower in both typical patients with type 2 diabetes and those with type 2 diabetes with a sedentary lifestyles (Chen et al., 2021). Therefore, a treatment strategy can be adopted for patients affected by type 2 diabetes to stimulate mitochondrial biogenesis. In skeletal muscle, the process of mitochondrial biogenesis requires PGC-1α, and mitochondrial content decreases when it is not activated (Chen et al., 2023). Physical exercise influences multiple metabolic pathways, mitigating the metabolic dysregulation observed in type 2 diabetes. Studies have shown that PGC-1α gene expression in skeletal muscle increases two hours after exercise and remains at a peak for up to 6 hours (Taylor et al., 2005). This expression was fully adapted after 54 days of continuous endurance exercise. As such, Bengal et al. (2020) concluded that PGC1-**α** leads to increased expression of glucose transporter type 4 (GLUT4), which subsequently enhances glucose consumption and significantly increases energy production in the muscle (Bengal et al., 2020). Furthermore, a relationship between decreased PGC-1**α** expression and increased fasting insulin levels and body mass index has been reported in individuals predisposed to diabetes (Park et al., 2017). Exercise activates various signaling pathways such as adenosine monophosphate kinase, mitogen-activated protein kinase, and increased calcium, which in turn increase the expression and activity of PGC-1α. This leads to a shift in muscle function from glycolytic to oxidative metabolism, thereby enhancing muscle endurance (Konopka et al., 2014). A study reported a significant increase in the protein content of PGC-1α and citrate synthase after 12 weeks of aerobic exercise in young, healthy subjects. This suggests that age does not influence changes in these two key markers of mitochondrial function and biogenesis. In this regard, Granata and his colleagues reported that exercise intensity is a crucial factor in exercise-dependent changes in the regulation of mitochondrial function and mitochondrial content (Granata et al., 2017).

Moreover, mitochondrial dysfunction has been repeatedly implicated in the development of insulin resistance, suggesting that defects in PGC-1α may contribute to this process. The observation that many PGC-1**α** target genes are downregulated in humans with diabetes further supports this idea (Rius-Pérez et al., 2020). In a study, an average decrease of 20 to 36% in PGC-1**α** mRNA expression was observed in the muscles of patients with DM and asymptomatic individuals with a family history of type 2 diabetes (Mootha et al., 2003).

Exercise significantly impacts PGC-1α mRNA expression (Heiat et al., 2020), while inactivity can decrease it. PGC-1α increases the expression of mitochondrial enzymes such as cyclooxygenase 3 (COX3) and the activity of carbohydrate and fat oxidation enzymes by upregulating the expression levels of nuclear respiratory factors 1 and 2 (Nrf1 and Nrf2) and estrogen receptor alpha (ERR-α) (Lira et al., 2010). Previous studies have shown that Nrf2 affects cellular bioenergy by modulating substrate availability and the oxidation efficiency of mitochondrial fatty acids (Holmström et al., 2013). PGC-1α and Nrf2 are closely related in the regulatory pathways and antioxidant response elements. Although

no direct signaling pathway has been identified, it is speculated that the pathways of these two transcription factors may influence each other, both responding to ROS. Additionally, Nrf2 regulates various mitochondrial enzymes that are also associated with PGC-1α activation (Dinkova-Kostova and Abramov 2015a). Nrf2 stands out as the primary regulator of the antioxidant cellular defense system. It achieves this by coordinating various responses specifically targeted towards neutralizing ROS (Kobayashi and Yamamoto 2006; Yavari et al., 2015). During activities with high intensity (above 90% VO2 max) or short duration (less than 420 seconds), there is a rapid increase in ROS formation, acidosis, and muscle fatigue (McGinley and Bishop 2017). These factors are recognized as major limiting factors in athletic performance, and various nutritional strategies have been investigated following World Anti-Doping Agency (WADA) guidelines.

Muscle fatigue can result from several causes, with the accumulation of hydrogen ions (H+) within the muscle cell being considered the primary source of fatigue during high-intensity short-term training. Consequently, dietary approaches using beta-alanine and sodium bicarbonate supplements have been employed to enhance intracellular and extracellular buffer capacity, thereby alleviating muscle fatigue during such exercises (Lancha Junior et al., 2015).

Sodium citrate is another nutritional strategy whose ergogenic potential has not been widely recognized since its introduction by Parry-Billings and MacLaren three decades ago (Parry-Billings and MacLaren 1986). Urwin et al. studied the effects of sodium citrate supplementation and demonstrated that, similar to bicarbonate, it is highly effective and causes minor digestive issues (Urwin et al., 2019). Subsequent research has supported these findings, noting that the peak concentration of bicarbonate ($HCO3^-$) for sodium bicarbonate occurs earlier, whereas with sodium citrate, the $HCO3^-$ peak is observed after 170 minutes. Like NaHCO3, sodium citrate enhances extracellular buffer capacity by indirectly raising intramuscular pH through facilitated H+ transport (Requena et al., 2005). Specifically, sodium citrate supplementation removes negatively charged citrate anions from plasma, decreasing plasma H+ while increasing $HCO3^-$ levels (Cunha et al., 2019) thereby improving buffering capabilities. Several studies have reported enhanced sports performance due to sodium citrate supplementation (Urwin et al., 2021). No studies have investigated PGC-1α expression and Nrf2 proteins with sodium citrate supplementation in diabetic rats after eight weeks of MICT training. We hypothesized that MICT would specifically increase the expression of PGC-1α and Nrf2 proteins, and that sodium citrate supplementation in diabetic rats could have a synergistic effect. Therefore, the purpose of this study was to investigate how two months of exercise combined with sodium citrate supplementation affects PGC-1α and Nrf2 expression in male diabetic rats.

Material and methods

Animal sample and groups

The Laboratory Animal Breeding Center of Tabriz University of Medical Sciences provided 45 three-month-old male Wistar rats weighing between 210 and 230 grams for the present study. To acclimatize the rats and minimize stress and physiological changes, they were housed under a 12-hour light-dark cycle (lights on from 4:00 am to 4:00 pm), at a temperature of 21° ± 3° C, and with humidity maintained between 45% and 55%. The cages were made of transparent autoclavable polycarbonate. Throughout the study period, all animals had ad libitum access to standard rat chow (pellets purchased from Isfahan Food Manufacturers Company) and water, which were measured and recorded accurately.

Upon arrival at the laboratory, the rats were randomly divided into five equal groups (N = 9 per group): (1) healthy control (C), (2) diabetic (D), (3) diabetes + exercise (DE), (4) diabetes + supplementation (DS), and (5) diabetic + exercise + supplementation (DSE), based on their body weights. All experimental procedures were conducted in accordance with the guidelines provided by the ethics committee at the University of Mohaghegh Ardabili, with the approval code IR.UMA.REC.1400.074, and followed the Principles of Laboratory Animal Care as per the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Sample size

To calculate the sample size using G*Power version 3.1.9.7 software, follow the analysis method using F-test in the ANOVA test a priori with a statistical power α err probability of 0.05, a power 1-β err probability of 0.80, a minimum effect size of 0.28, five groups, and two measurements. It has shown 82% sample power (i.e., actual

power) with 45 rats.

Diabetes induction

The rats underwent two weeks of high-fat diet intake once they had adapted to the laboratory surroundings, containing 45% fat, 21% protein, and 34% carbohydrates. The diet of the control group included 18% fat, 21% protein, and 61% carbohydrates. After two weeks, intraperitoneal (IP) injection of streptozotocin toxin (Sigma Aldrich Co., USA) at a dose of 35 mg/kg body weight dissolved in 0.1 M citrate buffer (pH = 4.5, IF) was administered after six hours of fasting (Zhang et al., 2008). An equivalent volume of physiological saline solution was injected into the healthy and diabetic control groups (no supplementation and no exercise) to maintain consistent conditions with the supplemented groups.

Sample collection

One week following diabetes induction, a drop of blood from the animal's tail vein was placed on a glucose assay kit (ARKRAY Inc., Japan). Blood glucose concentrations above 250 mg/dL were observed in the type 2 diabetic rats. Additionally, insulin levels in group D were compared with those in group C for further clarification. Hematoxylin and eosin staining at ×200 magnification was also used on the soleus muscle for additional certainty. Black lines in group D indicate infiltration of inflammatory cells in diabetic rats due to STZ.

Exercise program

Building upon prior research, this study employed a rigorous exercise program for the subjects. The rats underwent training sessions five days a week for eight consecutive weeks (Model 1050 LS Exer3/6; Columbus Instruments, USA). Each session consisted of continuous exercise on a treadmill at 70% of their maximum running speed (MRS) for 36 minutes.

To ensure proper acclimatization, a treadmill familiarization session was conducted a week before training commenced. This session took place on a treadmill set at a 0° slope and a comfortable speed of 15 meters per minute (m/min). Over the course of the acclimatization week, both the speed and incline of the treadmill were gradually increased. The speed rose by 2 m/min every two days, while the slope increased by 5° every two days. Additionally, the session duration grew by 2 minutes each day.

The MRS of the diabetic rats was measured two days after the induction of diabetes. The initial test began with a speed of 13 m/min on a 10° incline, and the rats ran at this pace for 5 minutes. Following this, the speed was progressively increased by 3.6 m/min every 2 minutes until the animals reached exhaustion. Throughout the training program, the MRS was assessed every 2 weeks to monitor progress. The average running speed during the daily exercise sessions was recorded at 24.5 ± 3.36 m/min, with a range of speeds observed between 17.9 m/min and 33.1 m/min (Chavanelle et al., 2017). (Chavanelle et al., 2017).

Sodium citrate supplement

For sodium citrate administration (JHD, Shantou, Guangdong, China), the designated rats were treated with a dose of sodium citrate (15 mmol/L) dissolved in drinking water for two months (Ou et al., 2015). It was administered to achieve plasma levels during activity, with sodium citrate given 3 hours before exercise (Urwin et al., 2021).

Western blot

Soleus muscle proteins were extracted using a radioimmunoprecipitation assay buffer containing 0.05 mM EGTA, 1% SDS, 0.1% cocktail antiprotease in Tris buffer (pH 8, 150 mM sodium chloride) (Roche, Mannheim, Germany). For this purpose, 100 mg of tissue in 500 µl

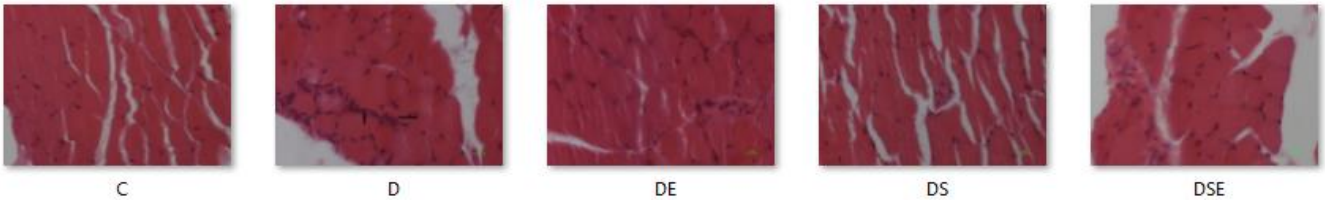

FIGURE 1. Hematoxylin and eosin staining at ×200 times

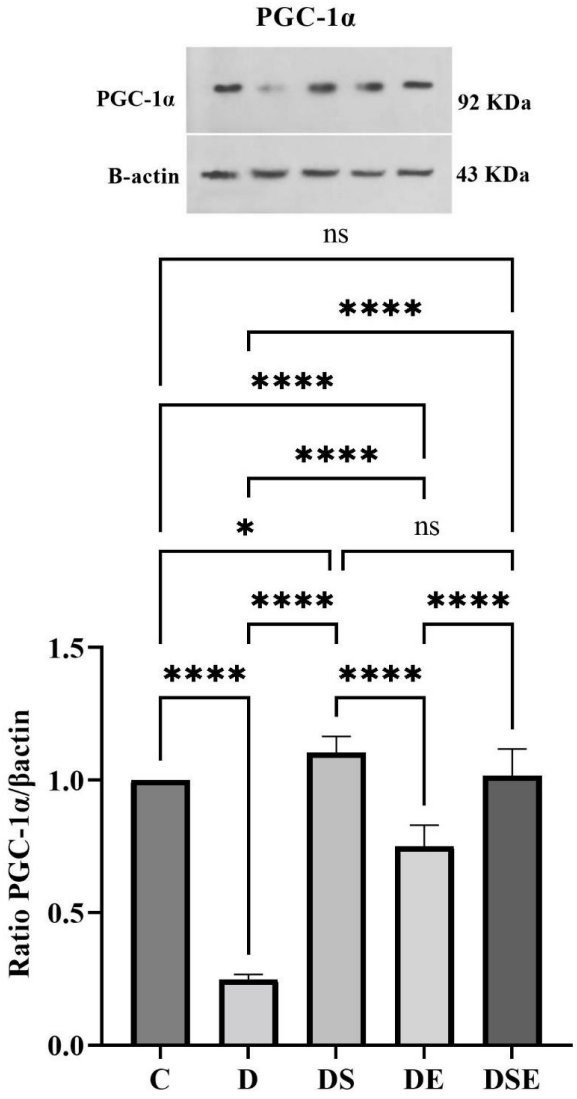

FIGURE 2. Investigation of changes in PGC-1α protein levels in different groups with ANOVA statistical test five equal-groups (N=9)
*= P ≤ 0.05
****= P ≤ 0.0001
C = control, D = diabetes, DS = diabetes + sodium citrate, DE = diabetes + exercise, DSE = diabetes + sodium citrate + exercise.

of antiprotease buffer was homogenized using a manual homogenizer and left for 4 hours at 4 °C. Subsequently, the homogenate was centrifuged in a refrigerated centrifuge at 12,000 rpm and 4 °C for 10 minutes. The supernatant was collected, and its protein concentration was determined using a microplate reader Mini Protean Tetra Cell Unit (Bio-Rad) at 595 nm wavelength. Finally, it was stored at -20 °C, and the obtained homogeneity was mixed with a loading buffer sample in a 1:1 ratio (50 mM Tris-HCl, 2% sodium bicarbonate, 0.005% bromophenol blue, 10% glycerol, 5% beta-mercaptoethanol). The samples were boiled for 5 minutes to denature the proteins thoroughly. SDS-polyacrylamide gel electrophoresis was used to separate the proteins, which were then transferred to a nitrocellulose membrane. The membrane was blocked for 1 hour in a solution containing 0.1% BSA in Tris-Buffered Saline (TBS) and 5% BSA in TBS-Tween 20 (TBST), followed by incubation with the primary antibody diluted 1:500. The following day, the membrane was incubated with secondary antibody for 1 hour at room temperature in 4% TBST. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system, and densitometry analysis was performed using Image J software (Kim 2017). Primary antibodies used were PGC-1α (ab54481, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA)

TABLE 1: Results of Tukey's post-hoc analysis for PGC-1α.

Groups	Comparisons	Difference (CI 95%)	p	Effect size (CI 95%)
C	**D**	0.75 (0.65 to 0.86)	**≤0.001**	1.03 (-1.47 to 2.83) L
	DE	-0.10 (-0.20 to 0005)	0.052	0.13 (0.203 to 0.317) T
	DS	0.25 (0.15 to 0.35)	**≤0.001**	0.35 (0.037 to 0.263) S
	DSE	-0.02 (-0.13 to 0.07)	0.930	0.03 (-0.91 to 0.051) T
D	**DE**	-0.85 (-0.96 to -0.75)	**≤0.001**	1.17 (-0.558 to 0.442) M
	DS	-0.49 (-0.60 to -0.39)	**≤0.001**	0.68 (-0.904 to -0.816) M
	DSE	-0.78 (-0.88 to -0.68)	**≤0.001**	1.07 (-0.851 to -0.709) M
DS	**DE**	-0.35 (0.46 to 0.25)	**≤0.001**	0.49 (0.289 to 0.431) S
	DSE	-0.28 (-0.38 to -0.18)	**≤0.001**	0.10 (-0.002 to 0.162) T
DE	**DSE**	-0.07 (-0.17 to -0.02)	0.251	0.39 (-0.370 to -0.190) S

Significant differences (P≤0.05) are highlighted in bold. C = control, D = diabetes, DS = diabetes + sodium citrate, DE = diabetes + exercise, DSE = diabetes + sodium citrate + exercise**,** T= trial, S=small, M=moderate, L= large, V=very large.

and NRF2 (sc-365949, Santa Cruz Biotechnology, Inc.). Beta-actin (Santa Cruz Biotechnology, Inc.) was used as a loading control.

Statistical analysis

The normal distribution of the data was assessed using the Shapiro-Wilk test. Differences among and within groups were analyzed using ANOVA with a Tukey post-hoc test. Effect sizes (ES) were reported using partial eta-squared () and Cohen's D. To interpret the effect size, the following criteria were employed: <0.2 = trivial, 0.2 to <0.6 = small effect, 0.6 to <1.2 = moderate effect, 1.2 to 2.0 = large effect, and >2.0 = very large effect. Statistical calculations were performed using SPSS, Version 27 (SSPS Inc., Chicago, IL).

Statistical significance was defined as P< 0.05. Graphs and tables were created using GraphPad Prism 9 software.

Results

PGC-1α Expression

A significant difference between the groups was observed in the expression of PGC-1α (F = 196.03; P<0.001; η_p^2= 1.36). Moreover, the study uncovered a significant difference in PGC-1α expression among the experimental groups. Group D exhibited an approximate 75.56% decrease (P=0.001) compared to the control groups. 75.56% decrease (P = 0.001) was seen in group D, a 10.23% decrease compared to the healthy group. Additionally, group DSE exhibited a non-significant 2.82% increase in PGC-1α protein expression compared to the control group. Moreover, the increased level of PGC-1α in group DSE was 3.3-fold lower than that in the supplementation group, which was statistically significant (P= 0.001). The results indicated that MICT training had the most significant effect on PGC-1α expression, and sodium citrate supplementation did not produce a synergistic effect (Figure 2).

* The difference among the groups were statistically significant (P≤0.05). C = control, D = diabetes, DS = diabetes + sodium citrate, DE = diabetes + exercise, DSE = diabetes + sodium citrate + exercise.

Additionally, the combination of two independent variables (DS) resulted in a significant reduction of approximately 96.27% (P = 0.001) compared to the diabetes group (P = 0.930) (Table 1).

Nrf2 Expression

A significant difference among the groups was observed in the expression of Nrf2 (F=2.239; P≥ 0.088; η_p^2= 0.24). The results showed that induction of diabetes decreases Nrf2 expression. However, group DSE did not show an increase in Nrf2 expression (Figure 3).

The expression of Nrf2 exhibited a non-significant decrease of about 27.20% in group D, 14.17% in group DE, 14.49% in group DS, and 13.97% in group DSE (Table 2).

TABLE 2: The results of Tukey's post-hoc analysis for Nrf2.

Group	Comparisons	Difference (CI 95%)	p	Effect size (CI 95%)
C	D	0.27 (0.008 to 0.53)	041،0	0.23 (0.061 to 0.449) S
	DE	0.14 (-0.12-0.40)	535،0	0.12 (0.072 to 0.228) T
	DS	0.14 (-0.11 to 0.40)	513،0	0.12 (0.037 to 0.263) T
	DSE	0.13 (-0.12 to 0.40)	0.548	0.12 (0.098 to 0.182) T
D	**DE**	-0.13 (-0.39 to 0.13)	0.612	0.11 (-0.372 to 0.092) T
	DS	-0.12 (-0.39 to 0.13)	0.513	0.11 (-0.387 to 0.107) T
	DSE	-0.13 (-0.39 to 0.13)	0.598	0.11 (-0.363 to 0.083) T
DS	DE	-0.003 (-0.26 to 0.26)	<0.999	0.002 (-0.137 to 0.137) T
	DSE	-0.005 (-0.26 to 0.25)	<0.999	0.001 (-0.131 to 0.111) T
DE	DSE	0.003 (-0.26 to 0.26)	<0.999	0.004 (-0.099 to 0.079) T

statistically significant differences ($p \leq 0.05$) are bolded. C = control, D = diabetes, DS = diabetes + sodium citrate, DE = diabetes + exercise, DSE = diabetes + sodium citrate + exercise, T= trial, S=small, M=moderate, L= large, V=very large.

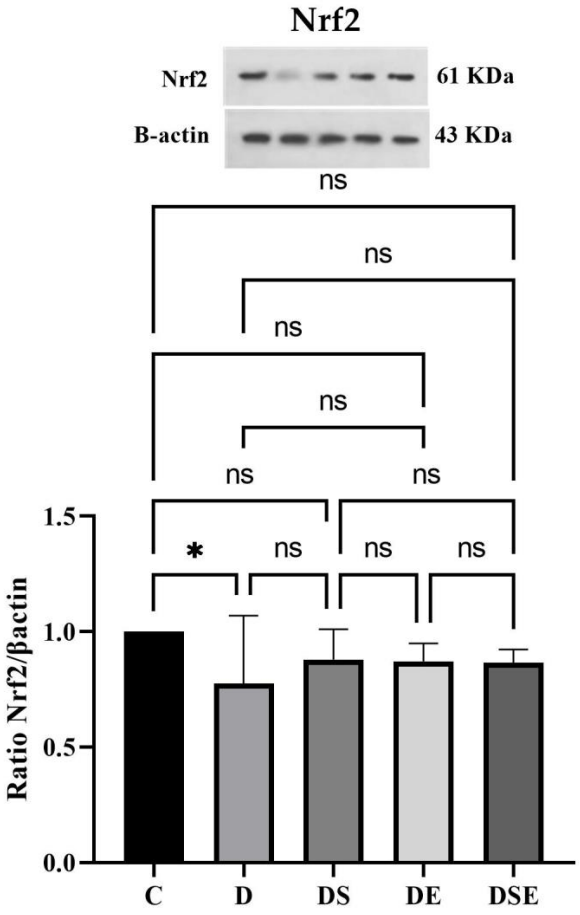

FIGURE 3. Investigation of changes in Nrf2 protein levels in different groups with ANOVA statistical test five equal-groups (N=9)
*= $P \leq 0.05$
C = control, D = diabetes, DS = diabetes + sodium citrate, DE = diabetes + exercise, DSE = diabetes + sodium citrate + exercise.

Discussion

This study aimed to investigate the impact of two months of sodium citrate supplementation combined with moderate-intensity continuous training on PGC-1α and Nrf2 expression in diabetic rats. The results indicated that diabetes induction significantly reduces PGC-1α expression in rats. This decrease in PGC-1α expression may impair skeletal muscle glucose uptake. Previous studies have demonstrated a significant association between PGC-1α levels and glucose absorption. Endoplasmic reticulum stress and oxidative stress suggest that disruption of a PGC-1α-dependent signaling pathway can impair GLUT4 production and glucose uptake (Raciti et al., 2010).

Furthermore, studies have shown that PGC-1α regulates GLUT4 expression and muscle displacement. At least two mechanisms mediate this process. Mora and Pessin (Mora and Pessin 2000) showed that myocyte-enhancer factor A2 (MEF2A) binds to the GLUT4 promoter and regulates its transcription in skeletal muscle. PGC-1α can enhance MEF2A expression by activating nuclear respiratory factor 1 (NRF1) (Ramachandran et al., 2008). Furthermore, this study revealed that MICT increases PGC-1α expression in diabetic male rats. Other studies have demonstrated that PGC-1α levels increase substantially during moderate-to-high-intensity endurance exercise, thereby enhancing its effect on GLUT4 expression (Pilegaard et al., 2003). Additionally, overexpression of MEF2C was found to be necessary but insufficient for GLUT4 transcription (Handschin et al., 2003). According to theory, PGC-1α may regulate GLUT4 expression through a MEF2-independent signaling pathway (Kramer et al., 2006). In skeletal muscle, AMPK activator (AICAR), either alone or in combination with insulin, increases the phosphorylation of Akt substrate 160 kDa (AS160) (Kramer et al., 2006). Rab proteins in GLUT4 vesicles become active through the phosphorylation of AS160, facilitating their movement to the plasma membrane. The present study demonstrated that AICAR-induced GLUT4 expression is dependent on PGC-1α (Suwa et al., 2014). However, it was found that 60 minutes of cycling did not increase PGC-1α levels (McGee and Hargreaves 2004). This suggests that low-intensity physical activities, such as 60 minutes of cycling, may not sufficiently stimulate PGC-1α expression. Therefore, it may be more effective to enhance PGC-1α expression through moderate to high-intensity activities.

Additional findings from the present study demonstrated that sodium citrate supplementation significantly increased the expression of PGC-1α in male diabetic rats. However, DSE did not produce an additional effect compared to DS alone. Sodium citrate ingestion reduced the interstitial accumulation of K+ released by muscles during intense exercise. The accumulation of K+ contributes to fatigue (Sundberg and Fitts 2019) and reduces muscle excitability (Nielsen et al., 2004a). It has also been suggested that an intermediate reduction of K^+ during exercise is associated with better performance (Nielsen et al., 2004b). One potential reason for the lack of synergism between sodium citrate supplementation and exercise could be attributed to the intensity of the exercises. Moderate-intensity exercises accumulate less lactic acid compared to high-intensity exercises, resulting in fewer buffering demands (Neufer 2018). Therefore, examining studies involving different exercise intensities, particularly high-intensity exercises, could provide researchers with more insights into the interaction between exercise and sodium citrate supplementation.

The present study revealed a significant suppression of Nrf2 expression in diabetic rats. This suppression has been linked to apoptosis and a reduced capacity for muscle regeneration (Narasimhan et al., 2014). However, acute physical activity triggers compensatory mechanisms despite the loss of Nrf2, such as the independent activation of antioxidant systems coordinated by other sensors like PGC1-α. Exercise enhances Nrf2 activity and the antioxidant cytoprotection system against oxidative stress damage (Gounder et al., 2012).

Despite two months of MICT along with sodium citrate supplementation, Nrf2 expression did not increase significantly. It appears that sodium citrate supplementation alone or in combination with MICT does not confer an obvious advantage for Nrf2 expression.

This study has certain limitations: Nrf2 was measured in total, and it is recommended to measure nuclear Nrf2 in future research. Further studies are needed to determine the effective exercise intensity and supplementation doses. Nonetheless, PGC-1α and Nrf2 are positively associated with modular pathways and antioxidant response components (Deng et al., 2020). Although a direct molecular pathway has not been identified, it is speculated that the pathways of these two transcription

factors are dependent on ROS and may influence each other (Ruhee and Suzuki 2020). Moreover, Nrf2 regulates various mitochondrial enzymes associated with PGC-1α activation (Dinkova-Kostova and Abramov 2015b). However, no direct relationship between them was observed during MICT activity. PGC-1α may modulate transcriptional activity affecting the expression of NRF-1, mitochondrial transcription factor A (TFAM) genes, and the regulation of mtDNA and nucleus-encoded proteins (Hood 2009). Physical activity activates PGC-1α in skeletal muscle through factors such as nitric oxide (NO), P38 AMPK gene, calcium calmodulin-dependent kinase (CaMK), and AMPK. PGC-1α then increases the expression levels of nuclear respiratory factors 1 and 2 (NRFs) and estrogen receptor alpha (ERR-α), enhances the expression of mitochondrial enzymes such as cyclooxygenase 8 (COX3), and boosts the activity of carbohydrate and fat oxidation enzymes (Halling and Pilegaard 2020). Finally, sodium citrate was administered in drinking water, which could lead to variability as some animals might consume more than others. It is suggested to use oral gavage in future studies for more controlled administration.

Conclusion

Overall, MICT and sodium citrate supplementation increased PGC-1α expression in this study. However, sodium citrate supplementation did not show a synergistic effect with MICT. Therefore, MICT and sodium citrate supplementation could be a potential treatment strategy for diabetes. In contrast, neither MICT alone nor in combination with sodium citrate supplementation showed a significant effect on Nrf2 expression in any group. Thus, further research investigating different training intensities and doses of sodium citrate supplements is necessary to increase Nrf2 expression.

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Informed Consent Statement

N/A.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

All associated experimental protocols were approved by the ethics code provided by the University of Mohaghegh Ardabili, committee.UMA.REC.1400.074, followed by the Principles of Laboratory Animal Care, per the European Communities Council Directive of 24 Nov 1986 (86/609/EEC).

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