To appear in Exercise Physiology and Performance (EPP)
Received: 2024/10/07 Revised: 2024/10/18 Accepted: 2024/10/30
DOI:

Relationship Between Nrf2/Keap1 Pathway and Exercise Performance in Hippocampal Tissue of Diabetic Rats

Safoura Alizade[1], Mohammad Faramarzi*[1]

1. Department of Exercise Physiology, Faculty of Sport Sciences, University of Isfahan, Isfahan, Iran
2. Department of Exercise Physiology, Faculty of Sport Sciences, University of Isfahan, Isfahan, Iran

Abstract

Introduction: Diabetes mellitus is a common metabolic disorder known for reducing antioxidant levels and concurrently increasing the production of free radicals. The Nrf2/Keap1/ARE signaling pathway may relate to insulin resistance in the brain. Exercise training may activate the production of reactive oxygen species (ROS) and boost antioxidant signaling pathways such as Nrf2/Keap1/ARE in the brain.

Methods: Fifteen male Wistar rats were divided into three groups: diabetic endurance training (DET, n=5), diabetic resistance training (DRT, n=5), and diabetic control (DC, n=5). Diabetes was induced using streptozotocin (30 mg/kg, i.p.) and a high-fat diet (55% fat, 31% carbohydrate, and 14% protein). The DET group underwent eight weeks of training at 75–60% of velocity at maximal oxygen uptake (vVO2max), while the DRT group performed resistance exercises at 60% of their maximum voluntary carrying capacity (MVCC), with the rats climbing a ladder 14–20 times per session, five days a week.

Results: In the DET group, Nrf2 expression correlated positively with MVCC3 ($r= 0.47$, $p<0.05$), whereas no significant correlations were found in the DRT group, and a negative correlation was observed in the DC group. Nrf2 and vVO2max showed a significant negative correlation at the second measurement ($r = -0.728$, $P = 0.002$), especially in the DC group ($r = -0.85$, $P < 0.01$). Keap1 levels exhibited weak to moderate positive correlations with vVO2max across groups ($r = 0.34$, $p = 0.58$) and significant positive correlations with MVCC parameters, particularly in the DRT group ($r = 0.92$, $p = 0.01$).

Conclusion: The analysis revealed varying correlations between Nrf2 and Keap1 levels with exercise performance metrics (vVO2max and MVCC parameters), with significant relationships observed only in specific experimental groups, highlighting the need for further investigation into the differential effects of these proteins on exercise outcomes.

Keywords: Nrf2/Keap1 Pathway, Diabetes, Endurance training, Resistance training, Aging

Introduction

Diabetes mellitus is a complex metabolic disorder associated with insulin metabolism and a range of metabolic disturbances characterized by hyperglycemia [1]. Hyperglycemia can lead to the overproduction of reactive oxygen species (ROS), which in turn reduces antioxidant levels and causes mitochondrial dysfunction in various organs, including the brain [2]. Recent research has demonstrated that oxidative stress contributes to the development of several metabolic and neurodegenerative diseases, such as diabetes, Alzheimer's disease (AD), and aging. The abnormal elevation of glucose levels in diabetes is responsible for the excessive production of ROS, which also contributes to insulin resistance (IR) and altered glucose tolerance [3]. Aging is a known risk factor for the onset of both diabetes and its complications [4].

The nuclear factor erythroid 2-related factor 2 (Nrf2) is a redox-sensitive protein that serves as a key regulator of oxidative stress. Nrf2 modulates the expression of numerous antioxidant and detoxification genes [5-7]. protecting cells from oxidative damage by binding to antioxidant response elements (AREs) to enhance the transcription of protective genes [8]. Under normal conditions, Nrf2 is sequestered in the cytosol by Kelch-like ECH-associated protein 1 (Keap1), where it is targeted for polyubiquitination. However, upon exposure to oxidative stress, Keap1 releases Nrf2, allowing it to translocate to the nucleus. A reduction in Nrf2 expression has been associated with worsened cognitive outcomes across a variety of conditions [9].

Exercise training activates ROS production through pathways like the electron transport chain and promotes antioxidant signaling, including the Nrf2 pathway in the brain [10]. Antioxidant signaling pathways, such as those involving superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), nuclear factor κB (NF-κB), and mitogen-activated protein kinases (MAPK), are activated in response to elevated ROS levels during endurance (aerobic) exercise training [11]. Nrf2 is also involved in energy metabolism, responding to increased ROS levels in the brain by activating antioxidant responses [12]. Consequently, exercise training can enhance Nrf2 activity in the brain, particularly in the elderly [13]. Moreover, resistance exercise training increases the levels of adenosine diphosphate (ADP) and adenosine monophosphate (AMP), which activate AMP-activated protein kinase (AMPK) through allosteric regulation, leading to the phosphorylation of Nrf2 and its accumulation in the nucleus, thereby boosting its antioxidant capacity [14, 15]. In one study, Nrf2 levels were significantly elevated in both young and older adults after a 30-minute cycling session at 70% of maximal oxygen uptake (vVO2max) intensity [16]. Emerging evidence underscores the important role of exercise in mitigating oxidative stress by enhancing Nrf2 activity and regulating the expression of antioxidant genes, as well as controlling ROS levels through cellular antioxidants. For instance, research has shown that

Nrf2 gene expression increases following speed-resistance exercise training, with the greatest increase observed in endurance exercise training [17].

This study hypothesizes that exercise training enhances the activity of antioxidant regulatory proteins, specifically Nrf2 and Keap1, in the hippocampal tissue of aged diabetic rats. The research aims to investigate the correlation between these regulatory proteins and key physiological markers of endurance and resistance exercise performance, specifically maximum voluntary carrying capacity (MVCC) and velocity at maximal oxygen uptake (vVO2max). By elucidating the relationship between oxidative stress modulation, exercise adaptations, and hippocampal health in diabetes-induced aging, this study seeks to provide valuable insights into potential therapeutic strategies for mitigating the adverse effects of diabetes and aging on neurocognitive function and physical performance.

Materials and Methods

Animals

The study protocol was approved by the Animal Care and Use Committee of Shahrekord University, Shahrekord, Iran (IR.SKU.REC.1399.001). Male Wistar rats were obtained from the Institute of Laboratory Animal Science at the Pasteur Institute of Iran, Tehran, Iran. All rats were 21 months old, with a mean body weight of 427 ± 44 g. The animals were housed under controlled conditions, with a room temperature of 20 ± 2°C and a 12:12-hour light-dark cycle. They had unrestricted access to food and water.

Experimental protocol

After acclimatization to their housing and the treadmill, 15 rats were randomly assigned to three groups: diabetic control (DC), diabetic endurance training (DET), and diabetic resistance training (DRT), with five rats per group. The rats in the exercise groups underwent strength training (60% of maximum voluntary carrying capacity [MVCC], 14-20 climbs) or endurance training (60-75% of velocity at maximal oxygen uptake [vVO2max]) five days a week for eight weeks. After training, the animals were anesthetized with a ketamine/xylazine mixture, and their hippocampi were extracted and stored on ice at -80°C for further analysis.

Induction of type 2 diabetes model

T2D was induced by HFD/low-dose STZ (manufactured by the Sigma Company) based on the protocol proposed by Zhang et al. [18] and Liu et al. [19]. The rats in the HFD group received HFD (55%, 31%, and 14% of energy from fat, carbohydrate, and protein, respectively; 5.2 kcal/g). The diets continued for eight weeks for three groups. Over week four, the rats in the group with HFD/STZ-induced T2D received treatment with low-dose STZ (Sigma-Aldrich, St Louis, MO, USA). All the rats also received an intraperitoneal injection of low-dose STZ (30 mg/kg, dissolved in 0.1 M sodium citrate buffer at pH 4.4). A week later, blood glucose tests were prepared with the use of a blood glucometer. The animals with blood glucose levels lower than 16.7 mmol/l then received a second injection of STZ (30 mg/kg). These diets continued after injections. Four weeks following the injections, the animals having blood glucose concentrations over 16.7 mmol/l were regarded to have diabetes and then chosen for more examinations [20, 21].

Maximum voluntary carrying capacity (MVCC)

The rats were first familiarized with a vertical climbing model (110 cm height, 2-cm grid, 85° inclination) without any additional overloads [22]. Briefly, after a seven-day familiarization period, each rat underwent a test to determine its maximum voluntary carrying capacity (MVCC). During this test, each rat began by climbing the ladder while carrying 75% of its body weight and was allowed to rest at the top of the ladder for 120 seconds. After each completed climb, 30 grams were added to the total carried mass. This procedure was repeated successively until the rat failed to climb the entire length of the ladder on three consecutive attempts. MVCC was defined as the highest load successfully carried by the rat during this protocol.

Resistance training protocols

The trained groups underwent eight weeks of ladder resistance training at 60% of their maximum voluntary carrying capacity (MVCC), five days per week. Each training session consisted of 14–20 ladder climbs. The MVCC test was repeated at three time points: after two weeks (MVCC-1), after four weeks (MVCC-2), and after eight weeks (MVCC-3) of training. Sedentary rats also underwent MVCC tests at the same time intervals as the trained animals, but remained in their cages without participating in the training protocol (**Table 1**).

TABLE 1 Resistance training protocol

Number of sessions/week	**Repetitions**	**Intensity (%)**	**Rest**	**Weeks**
5	14-20	60% MVC	1 min	8

Determining Velocity at Maximal Oxygen Uptake (vVO_{2max})

The rodent treadmill test was used to evaluate vVO2max through a series of ten 3-minute running stages. According to Leandro et al., the initial running speed was set at 0.3 km/h, with an increase of 0.3 km/h every 3 minutes (at a 0% slope). The vVO2max was determined at the stage where the rats could no longer maintain the running pace [23].

Endurance training protocols

The animals in the endurance training group were initially introduced to the treadmill and familiarized with running for one week. After a 48-hour rest period following the final familiarization session, exhaustive testing was performed to determine the exercise intensity [24]. The rodent treadmill was used to evaluate vVO2max through ten 3-minute running stages. According to Leandro et al., the initial running speed was set at 0.3 km/h, with a 0.3 km/h increase every 3 minutes at a 0% incline. The vVO2max was determined at the stage when the rats could no longer maintain the running speed [23].

During the eight-week exercise protocol, the animals followed an endurance training program. The training intensity was calculated based on the vVO2max. At the beginning of the endurance training (ET), the animals were trained at 40-50% of vVO2max for 5 minutes with a 0% incline for warm-up. The protocol consisted of both high-intensity and low-intensity training bouts. In the first week, the high-intensity bouts involved running at 60% vVO2max for 2 minutes; in the second week, the intensity increased to 65% vVO2max; in

the third week, it was set at 70% vVO2max, and by the fourth week, the intensity reached 75% vVO2max, continuing until the end of the training period. Low-intensity bouts involved 2 minutes of running at 40% vVO2max from the first to the third week, and at 30% vVO2max from the fourth to the eighth week. Additionally, the number of high-intensity interval bouts increased from two to eight repetitions from the first to the eighth week [25](**Table 2**).

TABLE 2 Endurance training protocol

weeks	**Warm-up and cooldown**	**Interval training**	**Training total time**
Week 1	40%–50% of S max	Maximum speed test: (mean = 34.4 m/ min) 4 Repetition with by 60% S max and 3 with by 40% S max	16 min
Week 2	40%–50% of S max	4 Repetition with by 65% S max and 3 with by 40% S max	24 min
Week 3	40%–50% of S max	6 Repetition with by 70% S max and 5 with by 40% S max	32 min
Week 4	40%–50% of S max	8 Repetition with by 75% S max and 7 with by 30% S max	40 min
Week 5-8	40%–50% of S max	Maximum speed test: mean = 40.5 m/min) 8 Repetition with by 75% S max and 7 with by 30% S max	

Western blot analysis

The rats' hippocampi were homogenized in lysis buffer, and the supernatants were collected by centrifugation at 4°C at 12,000 rpm for 10 minutes. Protein concentrations were quantified using the Bradford protein assay. Polyvinylidene fluoride (PVDF) membranes were cut to the size of the gel, activated by shaking in methanol for 1 minute, washed with distilled water, and then placed in a transfer buffer. The membranes were incubated in 2% skim milk for 2 hours at room temperature to block non-specific binding sites, followed by incubation with primary antibodies for 16-18 hours. The following day, the membranes were washed three times, each for 1 minute. Subsequently, HRP-conjugated secondary antibodies were applied at room temperature for 1 hour and 15 minutes. Reactive bands were visualized using a chemiluminescence (ECL) kit. Quantification of the bands was performed using Image Pro Plus (IPP) software.

Plasma Glucose and Insulin Measurements

Fasting glucose concentrations were measured at the beginning of the experiment using a blood glucose meter to confirm that the animals had euglycemia. Following the HFD/STZ-induced diabetes model, blood glucose levels were re-assessed to determine the onset of hyperglycemia (fasting blood glucose [FBG] ≥ 200 mg/dl). Plasma insulin levels were subsequently measured using an ELISA kit (Monobind Co., Cat No: 5825-300; California, USA) in animals that had fasted for 4 hours.

Statistical analysis

Statistical analyses were conducted using SPSS Statistics software (version 25). Data normality was assessed using the Kolmogorov-Smirnov test, and variance homogeneity was evaluated with Mauchly's sphericity test. One-way ANOVA was utilized to analyze insulin and glucose levels. For correlation analyses, Pearson correlation coefficients were calculated to evaluate the relationships between antioxidant protein levels (Nrf2, Keap1) and key physiological markers, including MVCC and vVO2max. Prior to correlation analyses, the

data were checked for linearity and the absence of significant outliers. Graphs were generated using GraphPad Prism software, and the significance level was set at $P \leq 0.05$.

Results

Changes in glucose and insulin of diabetic rats

The results of the one-way ANOVA test no significant differences in blood glucose concentration between groups were found throughout the study (F = 2.126, P = 0.115) (Table 3). However, insulin levels showed a significant difference (F = 4.360, P = 0.011). Rats treated with resistance training exhibited a significant reduction in insulin expression (P < 0.01).

variable	Group			P-value	F
	DC	DET	DRT		
Glucose (mg/dl)	385.5±184.55	343.66±140.01	263.00±160.26	0.115	2.126
Insulin (ng/ml)	2.08±0.98	0.6±0.68	0.09±0.08	0.011*	4.360

TABLE 3: Insulin and Serum Glucose levels after eight weeks of exercise training. Values are presented as mean ± SEM. Data were analyzed using one-way ANOVA.

Correlation between Nrf2 expression and MVCC Parameters

The correlation analysis between NRF2 levels and MVCC across three time points (MVCC1, MVCC2, and MVCC3) revealed varying degrees of associations depending on the experimental groups (DC, DET, and DRT). In the DET group, NRF2 levels demonstrated a moderate negative correlation with MVCC1 ($r=-0.43$, $p>0.05$, effect size= 0.18). Conversely, positive correlations were observed with MVCC2 ($r= 0.38$, $p>0.05$, effect size = 0.14) and MVCC3 ($r= 0.47$, $p<0.05$, effect size = 0.22), indicating a potential association at later time points, particularly MVCC3. For the DRT group, NRF2 levels showed weak positive correlations with MVCC1 ($r= 0.30$, $p>0.05$, effect size = 0.09) and MVCC2 ($r= 0.28$, $p>0.05$, effect size = 0.08), and a negligible negative correlation with MVCC3 ($r= -0.07$, $p>0.05$, effect size = 0.01). None of these correlations reached statistical significance, suggesting no meaningful relationship in this group.

The results highlight significant negative correlations in the DC group (notably at MVCC2, $r = -0.60$, $p < 0.05$, effect size = 0.36) and moderate positive correlations in the DET group (notably at MVCC3). No significant associations were detected in the DRT group. The variability of correlations across groups emphasizes the group- and time-dependent nature of the relationship between Nrf2 and MVCC. Further research is required to explore these trends.

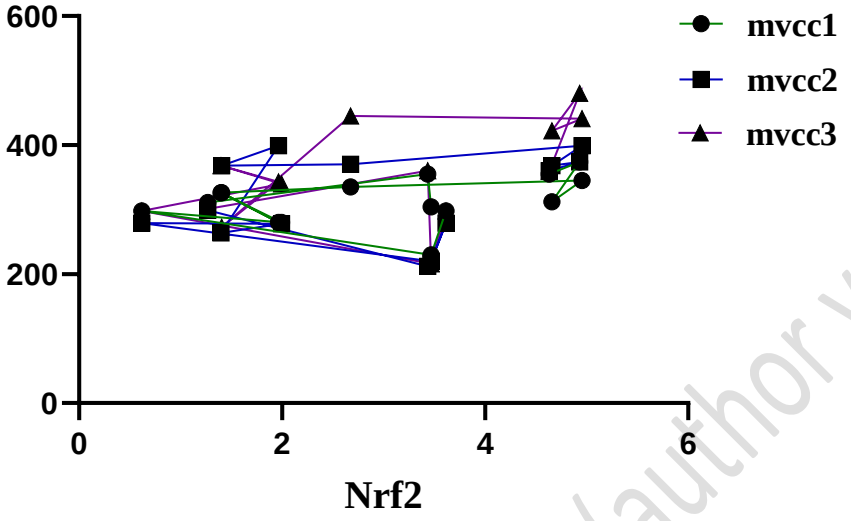

Figure 1. relationships (n=5) between Nrf2 expression and MVCC Parameters

Correlation between Nrf2 expression and vVO2max Parameters

The relationship between Nrf2 and vVO2max values at three measurement time points was analyzed. A weak positive correlation was observed between Nrf2 and the first measurement of vVO2max ($r = 0.182$, $P = 0.515$, effect size = 0.03), which was not statistically significant. A significant negative correlation was found between Nrf2 and the second measurement of vVO2max ($r = -0.728$, $P = 0.002$, effect size = 0.53), indicating an inverse relationship with large effect size. The third measurement of vVO2max showed a moderate negative correlation with Nrf2 ($r = -0.453$, $P = 0.09$, effect size = 0.20), which was not statistically significant. In the DC group, a significant negative correlation was observed between Nrf2 and vVO2max ($r = -0.85$, $P < 0.01$, effect size = 0.72), demonstrating a strong inverse relationship. These findings indicate a significant inverse relationship between Nrf2 and vVO2max, with notable differences in correlation patterns and effect sizes across experimental groups.

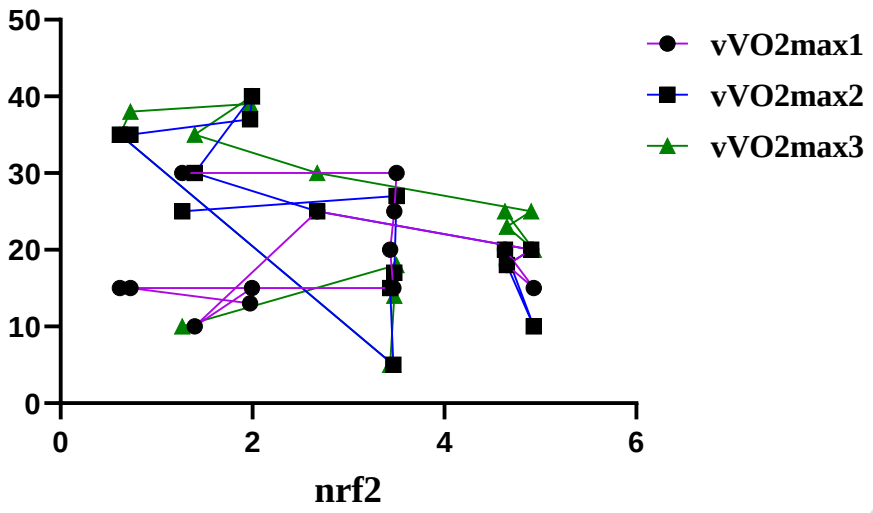

Figure 2. relationships (n=5) between Nrf2 expression and vVO2max Parameters

Correlation Between Keap1 and vVO2max Parameters

The relationship between Keap1 levels and vVO2max parameters (vVO2max1, vVO2max2, and vVO2max3) was analyzed across the three groups (DC, DET, and DRT). A weak positive correlation was observed between Keap1 and vVO2max1 ($r = 0.34$, $p = 0.58$, effect size = 0.12), indicating non-significance. Similarly, a weak negative correlation was identified between Keap1 and vVO2max2 ($r = -0.17$, $p = 0.75$, effect size = 0.03), and a weak positive correlation was found between Keap1 and vVO2max3 ($r = 0.20$, $p = 0.72$, effect size = 0.04), both of which were also not statistically significant. In the DRT group, Keap1 levels demonstrated a moderate positive correlation with vVO2max1 ($r = 0.53$, $p = 0.36$, effect size = 0.28), while weak positive correlations were observed with vVO2max2 ($r = 0.34$, $p > 0.05$, effect size = 0.12) and vVO2max3 ($r = 0.20$, $p > 0.05$, effect size = 0.04). Despite the presence of weak to moderate positive correlations in some instances, none of the correlations between Keap1 levels and the vVO2max parameters were statistically significant across the three groups. These findings suggest that further studies with larger sample sizes are needed to validate the observed trends.

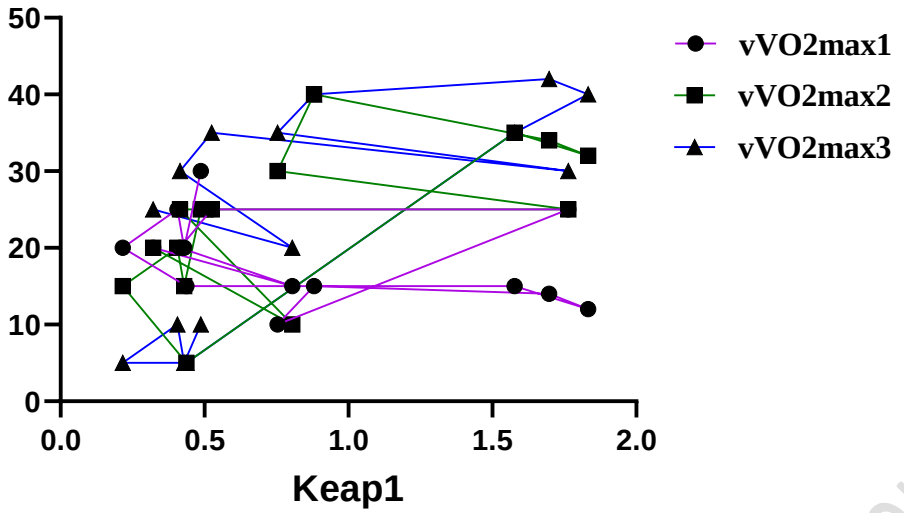

Figure 3. relationships (n=5) between Keap1 expression and vVO2max Parameters

Correlation Between Keap1 and MVCC Parameters

The relationship between Keap1 levels and MVCC parameters (mvcc1, mvcc2, and mvcc3) was assessed for each group (DC, DET, and DRT) using Pearson's correlation coefficients and their respective p-values. In the DC group, Keap1 levels exhibited a weak negative correlation with mvcc1 (r =-0.28, p=0.32, effect size = 0.08), a moderate positive correlation with mvcc2 (r = 0.60, p = 0.08, effect size = 0.36), and a weak negative correlation with mvcc3 (r = -0.31, p = 0.27, effect size = 0.10). In the DET group, Keap1 levels showed a moderate negative correlation with mvcc1 (r = -0.48, p = 0.15, effect size = 0.23), a weak negative correlation with mvcc2 (r = -0.33, p = 0.30, effect size = 0.11), and a weak negative correlation with mvcc3 (r = -0.28, p = 0.38, effect size = 0.08). None of these correlations were statistically significant. In the DRT group, Keap1 levels displayed a weak negative correlation with mvcc1 (*r*=-0.19, p= 0.60, effect size=0.04), a very strong positive correlation with mvcc2 (r = 0.92, p = 0.01, effect size = 0.85), and a moderate positive correlation with mvcc3 (r = 0.60, p = 0.10, effect size = 0.36). Among these, the correlation between Keap1 and mvcc2 was statistically significant ($p < 0.05$).

Overall, while correlations between Keap1 levels and MVCC parameters ranged from weak to strong across the groups, statistical significance was only observed for the relationship between Keap1 and mvcc2 in the DRT group. These findings suggest potential group-specific trends that warrant further investigation with larger sample sizes to confirm the observed patterns.

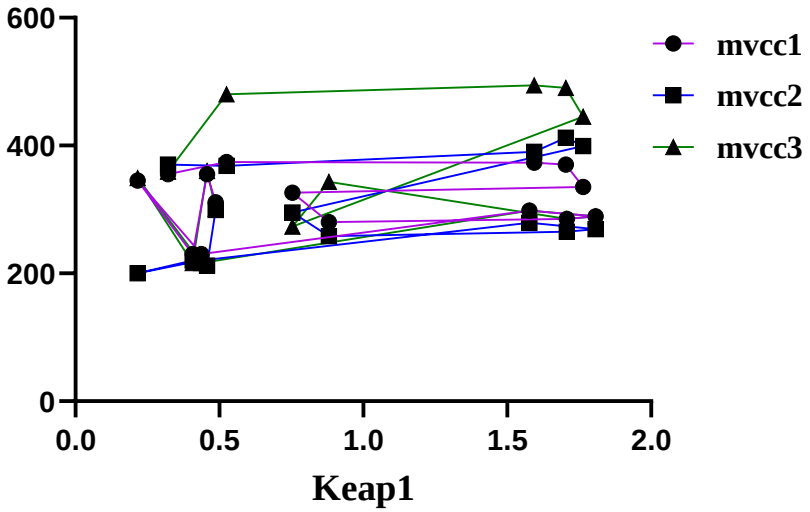

Figure 3. relationships (n=5) between MVCC expression and vVO2max Parameters

Discussion

Diabetes mellitus, as a common metabolic disorder, is associated with reduced antioxidant levels and increased production of free radicals, leading to exacerbated oxidative stress and cellular damage. These effects are particularly pronounced in tissues like the hippocampus, which play a critical role in cognitive functions. The Nrf2/Keap1/ARE signaling pathway serves as one of the primary antioxidant defense mechanisms in neuronal cells and may play a significant role in mitigating the deleterious effects of diabetes and aging.

Under physiological conditions, Keap1 inhibits Nrf2 by retaining it in the cytoplasm, preventing its activation. However, during oxidative stress, Nrf2 dissociates from Keap1 and translocates to the nucleus, where it activates genes associated with antioxidant response elements (ARE)[26]. In diabetic and aging conditions, this pathway may become dysregulated, reducing the hippocampus's capacity to counteract damage caused by reactive oxygen species (ROS)[27].

Exercise, as an effective non-pharmacological intervention, can activate antioxidant signaling pathways by inducing ROS production and enhancing Nrf2/Keap1 activity. The findings of the present study revealed differential effects of endurance and resistance training on the regulation of Nrf2 and Keap1. Specifically, a positive correlation between Nrf2 levels and some exercise performance indices (MVCC) was observed in the diabetic endurance training (DET) group, whereas no significant correlation was noted in the diabetic resistance training (DRT) group. Furthermore, a negative correlation between Nrf2 and vVO2max in the diabetic control (DC) group highlighted the detrimental impact of uncontrolled diabetes on this pathway. These results suggest that the Nrf2/Keap1 pathway may respond differently to various forms of exercise. Endurance training appeared to have a stronger effect on this pathway, potentially due to greater ROS production and subsequent activation of antioxidant responses. In contrast, resistance training might have more localized effects, requiring further investigations to elucidate the underlying mechanisms. The transition from a moderate negative correlation at MVCC1 to a significant positive correlation at MVCC3 in the DET group indicates that Nrf2 activation may progressively enhance muscular strength via

adaptive oxidative stress regulation. However, the inconsistent correlations in the DRT group suggest limited modulation of Nrf2-related antioxidant pathways by resistance training alone. Previous studies align with these findings, showing that exercise training improves hippocampal health by increasing blood flow and delivering more oxygen and nutrients [28]. Additionally, factors such as insulin-like growth factor 1 (IGF-1), testosterone, and leucine may further augment antioxidant signaling through the PI3K/Akt/mTOR pathways [29, 30]. The activation of Nrf2 can be further achieved by an increase in IGF-1, which permeates the brain following exercise training. IGF-1 may serve as a beneficial mediator of exercise training's impact on brain health, activating Nrf2 by enhancing Akt activity and reducing glycogen synthase kinase 3 beta (GSK-3b) activity [31]. Exercise training is, therefore, a key tool in managing chronic conditions associated with oxidative stress [32]. These results underscore the importance of exercise training as a key tool in managing chronic conditions associated with oxidative stress. Specifically, endurance training appears to play a pivotal role in enhancing systemic antioxidant defenses via the Nrf2/Keap1 pathway. For diabetic individuals, incorporating structured endurance exercise program into treatment protocols could improve not only oxidative stress regulation but also overall metabolic health.

Exercise training also induces a transient increase in reactive oxygen species (ROS) production through the upregulation of energy production, serving as the primary source of ROS [33]. Evidence suggests that exercise training intensity may play a role in further upregulating Nrf2 content [13]. Accordingly, Tutakhail et al. (2018) studied the effect of exercise training at three different intensities (14, 20, or 23.3 m/min, 60 min, 5 days per week) on the hippocampus and reported that Nrf2 content in the hippocampus increased after seven weeks with exercise training at intensities of 20 or 23.3 m/min, but no changes were observed in the group with 14 m/min exercise training intensity [13]. Moreover, Nemmar et al. (2018) found no differences in Nrf2 levels with lower intensity exercise training [34]. Camiletti-Moiron et al. (2015) had similarly demonstrated that resistance training could enhance brain Nrf2 through an increased abundance of oxidative/electrophilic stress [35]. The observed correlations between Nrf2 and vVO2max further highlight the intricate balance between antioxidant pathways and aerobic performance. The significant inverse relationship in the DC group suggests that higher Nrf2 levels may correspond to reduced aerobic capacity, potentially reflecting a trade-off between oxidative stress mitigation and performance adaptation. These findings underscore the complex interplay between redox homeostasis, exercise performance, and the distinct physiological responses elicited by varying exercise modalities. These findings highlight the need for personalized exercise protocols that consider the unique physiological and metabolic profiles of diabetic individuals. Further research is essential to elucidate these mechanisms and optimize exercise protocols for therapeutic applications.

Conclusion

This study demonstrates that endurance training has a more pronounced effect on modulating the Nrf2/Keap1 pathway and enhancing antioxidant defenses in the hippocampal tissue of aged diabetic rats, potentially improving muscular strength and aerobic performance. Conversely, resistance training showed limited effects on Nrf2 activation, suggesting that its

benefits may rely on alternative mechanisms such as PI3K/Akt/mTOR signaling pathways. These findings underscore the critical role of exercise, particularly endurance training, in mitigating oxidative stress and improving physical health in diabetic and aging populations.
Looking ahead, further research is necessary to explore the precise molecular mechanisms by which endurance training impacts oxidative stress regulation in the brain and other organs. Additionally, future studies should consider the long-term effects of exercise training across different intensities and durations, as well as potential synergistic interactions between endurance and resistance training. Moreover, clinical trials with larger sample sizes and diverse diabetic populations are essential to confirm these findings and to establish practical exercise guidelines for diabetes management. Ultimately, exercise training, especially endurance exercise, appears to be a promising non-pharmacological intervention that can enhance antioxidant capacity, alleviate oxidative stress, and improve both physical and cognitive health in individuals with chronic metabolic disorders such as diabetes.

Acknowledgments
The authors, hereby, appreciate the authorities of the Vice Chancellor's Office for Research and Postgraduate Studies of Shahrekord University, Shahrekord, Iran, for their help to fulfill this study.

Declarations

Ethics approval and consent to participate
This study was conducted according to the Ethical principles of Shahrekord University Animal Ethics Committee (Reference Number: IR.SKU.REC.1399.001). All the experiments were conducted in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Consent for publication
Not applicable.

Competing interests
There was no conflict interest.

References

1. Ma, N. and Y. Zhang, *Effects of resveratrol therapy on glucose metabolism, insulin resistance, inflammation, and renal function in the elderly patients with type 2 diabetes mellitus: A randomized controlled clinical trial protocol.* Medicine, 2022. **101**(32).
2. Pu, D., et al., *Protective effects of sulforaphane on cognitive impairments and AD-like lesions in diabetic mice are associated with the upregulation of Nrf2 transcription activity.* Neuroscience, 2018. **381**: p. 35-45.
3. Bhatti, J.S., et al., *Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives.* Free Radical Biology and Medicine, 2022.
4. Tanwar, A., K. Gulati, and A. Ray, *Neuroprotective Effect of Withania Somnifera Root Extract In Type 2 Diabetes Mellitus Induced Cognitive Impairment And Neurodegeneration In Rats.* Asian Journal of Pharmaceutical Research and Development, 2022. **10**(4): p. 47-54.
5. Kang, T.-C., *Nuclear factor-erythroid 2-related factor 2 (Nrf2) and mitochondrial dynamics/mitophagy in neurological diseases.* Antioxidants, 2020. **9**(7): p. 617.
6. Lee, K.H., M. Cha, and B.H. Lee, *Neuroprotective effect of antioxidants in the brain.* International journal of molecular sciences, 2020. **21**(19): p. 7152.

7. Liu, Y., J. Deng, and D. Fan, *G-Rh4 improves pancreatic β-cells dysfunction in vivo and in vitro by increased expression of Nrf2 and its target genes.* Food and Chemical Toxicology, 2021. **148**: p. 111925.
8. Zweig, J.A., et al., *Loss of NRF2 accelerates cognitive decline, exacerbates mitochondrial dysfunction, and is required for the cognitive enhancing effects of Centella asiatica during aging.* Neurobiology of Aging, 2021. **100**: p. 48-58.
9. Cai, M., et al., *The Molecular Mechanisms of Excessive Hippocampal Endoplasmic Reticulum Stress Depressing Cognition-related Proteins Expression and the Regulatory Effects of Nrf2.* Neuroscience, 2020. **431**: p. 152-165.
10. Kasai, S., et al., *Regulation of Nrf2 by mitochondrial reactive oxygen species in physiology and pathology.* Biomolecules, 2020. **10**(2): p. 320.
11. Wang, C., et al., *Oxidative parameters in the rat brain of chronic mild stress model for depression: relation to anhedonia-like responses.* The Journal of membrane biology, 2012. **245**(11): p. 675-681.
12. Done, A.J. and T. Traustadóttir, *Nrf2 mediates redox adaptations to exercise.* Redox biology, 2016. **10**: p. 191-199.
13. Tutakhail, A., et al., *Induction of brain Nrf2-HO-1 pathway and antinociception after different physical training paradigms in mice.* Life sciences, 2018. **209**: p. 149-156.
14. Oakhill, J.S., et al., *AMPK is a direct adenylate charge-regulated protein kinase.* Science, 2011. **332**(6036): p. 1433-1435.
15. Joo, M.S., et al., *AMPK facilitates nuclear accumulation of Nrf2 by phosphorylating at serine 550.* Molecular and cellular biology, 2016. **36**(14): p. 1931-1942.
16. Done, A.J., et al., *Exercise-induced Nrf2-signaling is impaired in aging.* Free Radical Biology and Medicine, 2016. **96**: p. 130-138.
17. Eynon, N., et al., *Interaction between SNPs in the NRF2 gene and elite endurance performance.* Physiological genomics, 2010. **41**(1): p. 78-81.
18. Zhang, M., et al., *The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model.* Experimental diabetes research, 2008. **2008**.
19. Liu, Z., et al., *Antidiabetic effects of malonyl ginsenosides from Panax ginseng on type 2 diabetic rats induced by high-fat diet and streptozotocin.* Journal of Ethnopharmacology, 2013. **145**(1): p. 233-240.
20. Qian, C., et al., *High-fat diet/low-dose streptozotocin-induced type 2 diabetes in rats impacts osteogenesis and Wnt signaling in bone marrow stromal cells.* PLoS One, 2015. **10**(8): p. e0136390.
21. de Bem, G.F., et al., *Antidiabetic effect of Euterpe oleracea Mart.(açai) extract and exercise training on high-fat diet and streptozotocin-induced diabetic rats: A positive interaction.* PLoS One, 2018. **13**(6): p. e0199207.
22. Singulani, M.P., et al., *Effects of strength training on osteogenic differentiation and bone strength in aging female Wistar rats.* Scientific reports, 2017. **7**: p. 42878.
23. Leandro, C.G., et al., *A program of moderate physical training for Wistar rats based on maximal oxygen consumption.* Journal of strength and conditioning research, 2007. **21**(3): p. 751.
24. PITHON-CURI, T.N.C., *Aprogram Of Moderate Physical Training For Wistar Rats Based On Maximal Oxygen Consumption.* Journal of strength and conditioning research, 2007. **21**(3): p. 000-000.
25. Nourshahi, M., et al., *Effect of 8 weeks endurance training on serum vascular endothelial growth factor and endostatin in wistar rats.* Koomesh, 2012. **13**(4): p. 474-479.
26. Egbujor, M.C., et al., *Activation of Nrf2 signaling pathway by natural and synthetic chalcones: A therapeutic road map for oxidative stress.* Expert review of clinical pharmacology, 2021. **14**(4): p. 465-480.

27. Yu, C. and J.-H. Xiao, *The Keap1 - Nrf2 system: a mediator between oxidative stress and aging.* Oxidative Medicine and Cellular Longevity, 2021. **2021**(1): p. 6635460.
28. Tao, J., et al., *Mind-body exercise improves cognitive function and modulates the function and structure of the hippocampus and anterior cingulate cortex in patients with mild cognitive impairment.* NeuroImage: Clinical, 2019. **23**: p. 101834.
29. Lane, M.T., et al., *Endocrine responses and acute mTOR pathway phosphorylation to resistance exercise with leucine and whey.* Biology of sport, 2017. **34**(2): p. 197-203.
30. Schoenfeld, B.J., et al., *Resistance training volume enhances muscle hypertrophy but not strength in trained men.* Medicine and science in sports and exercise, 2019. **51**(1): p. 94.
31. Rahimian, R., P. Cordeau Jr, and J. Kriz, *Brain response to injuries: when microglia go sexist.* Neuroscience, 2019. **405**: p. 14-23.
32. Pedersen, B.K. and B. Saltin, *Exercise as medicine–evidence for prescribing exercise as therapy in 26 different chronic diseases.* Scandinavian journal of medicine & science in sports, 2015. **25**: p. 1-72.
33. Liochev, S.I., *Reactive oxygen species and the free radical theory of aging.* Free radical biology and medicine, 2013. **60**: p. 1-4.
34. Nemmar, A., et al., *Exercise training mitigates water pipe smoke exposure-induced pulmonary impairment via inhibiting NF-κB and activating Nrf2 signalling pathways.* Oxidative Medicine and Cellular Longevity, 2018. **2018**.
35. Aranda, P., et al., *High-protein diet induces oxidative stress in rat brain: protective action of high-intensity exercise against lipid peroxidation.* Nutricion hospitalaria, 2015. **31**(2): p. 866-874.