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Cardioprotective effects of aerobic training in diabetic rats: Reducing cardiac apoptotic indices and oxidative stress for a healthier heart

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OriginalArticle

Abstract

BACKGROUND: The present study evaluated the effects of aerobic training with variable intensities on apoptotic indices of cardiac tissue in fatty diabetic rats.

METHODS: Twenty-four male Wistar rats were randomly divided into non-diabetic (ND, n=8), trained diabetic (TD, n=8), and control diabetic (CD, n=8) groups. Following a high-fat dietary regimen, type 2 diabetes was induced by streptozotocin, with blood glucose levels above 300 mg/dL considered indicative of diabetes. The TD group underwent aerobic exercise five times a week for six weeks. Subsequently, measurements were taken for left ventricular end-diastolic (LVEDV) and end-systolic volumes (LVESV), ejection fraction (EF%), catalase, caspase-9, P53, glucose, insulin, and HOMA-IR.

RESULTS: Aerobic training led to a significant decrease in blood glucose levels ($P < 0.01$), caspase-9 ($P < 0.05$), HOMA-IR ($P < 0.05$), and P53 expression ($P < 0.001$) compared with the CD group. LVEDV and LVESV decreased significantly ($P < 0.05$ for both), while LVEF increased significantly ($P < 0.05$). Catalase activation showed an insignificant increase in the TD group pre- to post-training compared to CD.

CONCLUSION: Incremental aerobic exercise training (6 weeks) may exert a cardioprotective effect in diabetic rats by reducing apoptosis and oxidative stress indices, while simultaneously increasing aerobic fitness and reducing body weight.

Keywords: Physical Activity; Diabetes; Apoptosis; Oxygen Consumption; Rat

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Introduction

Diabetes mellitus is a metabolic disorder that is rapidly growing and emerging as a public health issue due to its high prevalence rate and contribution to morbidity and mortality. Diabetes mellitus induces risk factors for cardiovascular disease that eventually lead to heart failure[1]. Diabetic Cardiomyopathy (DC) is a primary pathological factor contributing to heart disease, characterized by left ventricular (LV) diastolic and systolic dysfunction in the absence of hypertension[2]. Various molecular and metabolic factors, such as insulin resistance, reactive oxidative species, fatty acids, and inflammation, have been identified as contributing factors in DC[3-5].

In reviewing the literature related to DC, hyperglycemia caused by insulin resistance has been reported to be the main cause of cardiac dysfunction[6,7]. Endothelial cells, unable to limit glucose transport, are more vulnerable to the toxic effects of hyperglycemia[1]. Following hyperglycemia, there is an elevation in reactive oxygen species in the diabetic heart, which seems to be counterbalanced by the promotion of antioxidant defense through enhanced activation of enzymes such as

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Catalase, Glutathione Peroxidase, and Superoxide Dismutase[1,8-10]. Recent studies have demonstrated that antioxidant defense is also induced by voluntary physical training[1,9, 11]. However, the deleterious effects of metabolic dysfunctions following diabetes stimulate both extrinsic and intrinsic apoptotic pathways[3].

Apoptosis is a programmed cell death process necessary for the physiological balance of cells[12]. In the extrinsic apoptotic pathway, inflammation following hyperglycemia leads to the binding of tumor necrosis factor-α and FAS Cell Surface Death Receptor (FAS) to their respective receptors to trigger cell death[13]. Consequently, cardiomyocyte-induced Caspase 9 activates the apoptotic pathways leading to rapid and irreversible cell death[12], which decreases the contractile function of the heart and ultimately promotes cardiac remodeling[14].

During the intrinsic pathway, hyperglycemia leads to the release of a group of mitochondrial proteins such as cytochrome C (Cyto C), Bcl-2 Associated X-protein (Bax), FAS, and P53 into the cytosol, forming a complex that serves as a platform to activate Caspase-9 and Caspase-3 as downstream factors of apoptosis[15]. The rate of activation of downstream Caspases (Caspase-9 and Caspase-3), and the inclusion of proteins such as B-cell lymphoma 2 (Bcl-2) and BclXL (B-cell lymphoma-extra-large) that inhibit apoptosis, depend on changes in the mediators related to gene and protein expressions of the aforementioned factors[3,4,12,16,17].

Among these factors, P53 plays an essential regulatory role in mitochondria and metabolism in diabetic cardiomyopathy in cells under pathological conditions[18,19]. A growing body of evidence has demonstrated that inhibition of P53 prevents cardiac apoptosis during the initial stages of diabetes, attenuates cell degradation induced by diabetes, and improves both angiogenic and glycolytic defects[20]. Reports also show that P53 is a regulatory factor responsible for cardiovascular adaptation to aerobic training[19]. However, the potential mechanisms underlying these effects remain largely elusive.

In addition to its positive effects on insulin sensitivity, glycemic control, glucose uptake, cardiac structure, and function, aerobic training is considered a potential positive intervention recommended for diabetic patients[21-26]. In this context, the molecular processes that might be positively or negatively affected by aerobic training in diabetic cardiomyopathy are increasingly considered. Although several aerobic training protocols have been introduced as effective approaches in healthy individuals[27-31] and people with medical conditions, there is significant diversity among the utilized aerobic training schedules, and the adaptation of oxidative stress depends on the intensity, duration, and type of recruited activity.

Therefore, the purpose of the present study was to address the probable effects of aerobic training on aerobic capacity, insulin resistance, and LV catalase, caspase-9, and P53 activity as regulators of diabetic cardiomyopathy in type 2 diabetic fatty rats. We hypothesized that aerobic training might reduce cardiac apoptosis in trained rats with type 2 diabetes via the induction of efficient enzyme and protein production.

Materials and Methods

Figure 1 presents a schematic of the testing sequence and protocols. Twenty-four male rats (10 weeks old; 170-190 g) were used in this experiment. The rats were housed in standard rodent cages with dimensions of 25×27×42 cm within a clean and well-ventilated room at the University of Tehran's animal laboratory. The environment was maintained at a temperature of 22.1°C and adhered to a 12:12 light/dark cycle. The animals had unrestricted access to a standard diet and water. The experimental research was approved by the Animal Ethics Committee of Shahid Rajaee University of Tehran, with the reference number IR.SRTTU.SSF, 2020.129. Furthermore, all experiments were conducted in accordance with the guidelines prescribed by the Institutional Animal Care and Use Committee.

To ensure unbiased allocation, the rats were randomized and divided into three groups (each of 8): healthy non-diabetic (ND), high-fat diet groups including control diabetic (CD), and trained diabetic (TD). In the healthy control group, rodent-specific normal food was freely provided; but in the high-fat diet group, rats were given a high-fat diet for 4 weeks, containing 55% of total fat energy (derived from animal fat oil), 10% protein, and 35% carbohydrate (freely available). After 4 weeks on a high-fat diet, type 2 diabetes was induced by injecting streptozotocin (STZ) dissolved in sodium citrate buffer with pH = 4.2, at a dose of 40 mg/kg intraperitoneally[1]. Three days after STZ injection, blood glucose levels were evaluated, and values above 300 mg/dL were considered indicative of diabetes.

Rat height and weight were measured after a period of receiving a high-fat diet, and rats recognized as obese were matched based on body weight and fasting glucose. They were then randomly divided into two groups: diabetic aerobic exercise training and control group. For the remaining days of the study, the high-fat diet was switched to a normal diet.

Familiarization and Speed in VO_{2max} Test

After STZ-induced type 2 diabetes, the rats in the exercise group engaged in aerobic activity for 6 weeks. Seven days before the start of the experiment, the rats in the TD group participated in a familiarization training session on a horizontal treadmill (BIOSEB, Vitrolles, France), which included 10 minutes of running at a speed of 10 m/min. Before the beginning of the training period, running speed at maximal oxygen consumption (VO_{2max}) was evaluated by an incremental test. The test began with a 10-minute warm-up at a speed of 5 m/min. Then, the running speed increased by 1.8 m/min every 2 minutes until the animals could no longer continue running despite an increase in speed. The correlation between running velocity and VO_{2max} was previously investigated and validated by Hoydal et al. (2007), and the highest running speed was recorded as the speed at VO_{2max} [32]. Subsequently, the animals participated in a running program for 6 weeks, with 5 sessions per week.

Training protocol

The treadmill running program began at 65% of running speed in VO_{2max} on first week and progressively increased to 85% of running speed in vVO_{2max} at week 6 (65,70,70,75,75,85% running speed in VO_{2max}, from 1st to 6th week, respectively) 5 sessions per week. Also, the training duration progressively increased by 1 min/session. During each training session, following a 3-minute warm-up at a speed of 7 m/min, the speed was increased by 1.8 m/min every minute until the desired speed for that session was achieved. After finishing the exercise, the speed was continuously reduced to reach the initial speed. Subjects repeated the incremental test at the end of 3rd week to set running speed in VO_{2max} according to new VO_{2max} capability. Running speed in VO_{2max} and body weight was also measured again at the end of the workout.

Echocardiography

Rats underwent echocardiography 24 hours after the final training session, following the protocol outlined in our previous study[21]. To summarize the procedure briefly, rats were anesthetized using a Ketamine/Xylazine combination (1:10 ratio) and positioned in a left lateral decubitus position. M-mode and two-dimensional echocardiography (ECO) images were acquired using a 10-MHz probe from a GE-Vingmed Ultrasound system based in the USA. Left Ventricular End Diastolic Volume (LVEDV), Left Ventricular End Systolic Volume (LVESV), and the percentage of Ejection Fraction (EF %) were measured. The procedure was conducted by an operator who was blinded to the genotype, using specialized software (EchoPac v113; GE Healthcare).

Surgical Preparation and Metabolic Assessment

The animals received intraperitoneal anesthesia with pentobarbital sodium (65 mg/kg) 48 hours after the completion of the last training session. Subsequently, 2 ml of blood was collected from the left ventricle, and the heart was excised. The LV myocardium was

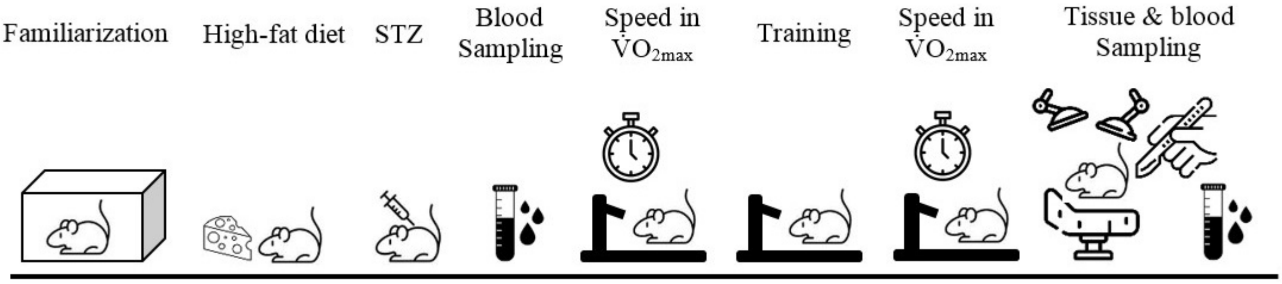

Figure 1. The timeline and schematic protocol of the experiment

separated from the heart, rapidly frozen in liquid nitrogen, and then stored at −80°C for later analysis of protein expression changes. To separate plasma, blood samples were centrifuged for 15 minutes at 3,000 g, and plasma samples were stored in 2 ml microtubes at −80°C for the measurement of fasting insulin and glucose levels. The insulin resistance index was calculated using the following equation:

HOMA-IR = (insulin * glucose) / 22.5

Plasma glucose levels were determined using the glucose oxidase method, and levels of insulin, Catalase, and Caspase-9 were measured using ELISA kits (Roche AS, Indianapolis, IN)[33,34].

P53 Measurement and Western Blotting

Total protein was extracted from lysates obtained from the left ventricular myocardium after centrifugation at 14,000 g for 10 minutes at 4°C. The protein was separated by SDS-PAGE (12 percent) and transferred onto a nitrocellulose membrane. The membrane was then blocked with 5% dry milk and incubated overnight in a solution containing primary antibodies. In this study, Anti-P53 antibody and Anti-Beta actinin antibody (rabbit/rat; polyclonal; 1:1,000 dilution; #2772; Cell Signaling Technology, Inc., Danvers, MA, USA) were used as primary antibodies. The following day, the membrane was incubated with the secondary antibody (goat/rabbit; polyclonal; 1:1,000; A0208; Beyotime Institute of Biotechnology) for 30 minutes. Blots were imaged using a Bio-Rad gel imaging system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Subsequently, band densities were quantified using Quantity One software, version 4.5 (Bio-Rad Laboratories, Inc.).

Statistical analysis

Statistical analyses were performed using SPSS software version 14.0 (IBM Corp., Chicago, IL). Descriptive data are presented as mean ± SD. A two-factor mixed analysis of variance (ANOVA) with the between-factor "group" (ND, TD, and CD) and repeated factor "trial" (baseline, week 3, and post-test) was conducted to determine if changes in measured variables over time differed by intervention. The Shapiro-Wilk test was used to examine the normality of distribution, and Levene's test assessed the homogeneity of variances. Mauchly's W test confirmed sphericity, indicating that the sphericity assumptions for repeated measures ANOVA were met. The alpha level was set at 0.05.

Results

Table 1 presents the main effects and time-regimen interactions for the measured variables. At baseline, weight, glucose, and HOMA-IR levels were significantly higher in the TD and CD groups compared to the ND group. Over time, a significant increase in weight was observed in the ND and CD groups. However, no significant time-regimen interaction was detected for this variable.

At baseline, a significant between-group difference was observed in vVO_{2peak}, with the ND and TD groups showing higher values compared to the CD group. A significant time effect and time-regimen interaction ($p < 0.05$) were noted for this variable. Specifically, TD led to a significant change in vVO_{2peak} from pre-training to week 3 and from week 3 to post-test (Table 1). Moreover, the increase in this variable over the first three weeks was significantly greater in the TD group compared to the ND ($P = 0.02$) and CD ($P = 0.00$) groups. Additionally, the TD group experienced a significantly greater change in vVO_{2peak} from pre- to post-training compared to the CD group ($P = 0.01$).

At baseline, week 3, and post-test, both TD and CD groups exhibited notably higher glucose levels compared to the ND group (Table 1). A significant time effect and time-regimen interaction ($P < 0.05$) were observed in glucose levels. Specifically, the TD group experienced a significant reduction in glucose levels from pre- to post-training, with the magnitude of this change significantly greater in TD than in the CD ($P = 0.66$) and ND ($P = 0.27$) groups.

No notable between-group differences were observed for insulin levels at baseline. However, a significant time effect and time-regimen interaction ($p < 0.05$) were detected in insulin levels. Specifically, the TD group showed a significant change in insulin levels from pre- to post-training. Additionally, the change in this variable over the initial three weeks was significantly greater in the TD group compared to the ND group ($P = 0.03$). Moreover, the TD group experienced a greater change in insulin levels from pre- to post-training ($P = 0.04$) compared to both the CD ($P = 0.53$) and ND ($P = 0.07$) groups.

HOMA-IR levels in the TD and CD groups were significantly higher than those in the ND group at all three time points ($P < 0.05$). A significant time effect and time-regimen interaction were observed

Table 1. Body weight, Glucose, Insulin level, and insulin resistance index (HOMA-IR) in untrained Non-Diabetic (ND), Control Diabetic (CD) and Trained Diabetic (TD) rats in pre-test, at the end of 3th week and post-test (Mean ± SD)

Variables	Group ND	TD	CD
Weight (g)			
Pre	227 ± 23	256 ± 28‡	254 ± 39‡
Week 3	240 ± 19	249 ± 30	251 ± 29
p	P = 0.00	P = 0.29	P = 0.48
Post	245 ± 27*	243 ± 32	257 ± 34*
p	P = 0.00	P = 0.12	P = 0.66
vVO_{2peak} (m/min)			
Pre	21.23 ± 4.9†	20.16 ± 5.8†	18.87 ± 6.6
Week 3	20.92 ± 6.1	23.8 ± 3.7*’⁑’†’‡	19.13 ± 5.1
p	P = 0.69	P = 0.04	P = 0.74
Post	20.69 ± 4.7	27.6 ± 5.1*’†	19.41 ± 5.4
p	P = 0.63	P = 0.00	P = 0.61
Glucose (mg/dL)			
Pre	97 ± 8	449 ± 54‡	463 ± 81‡
Week 3	105 ± 5	432 ± 41‡’⁑	459 ± 69‡
p	P = 0.46	P = 0.37	P = 0.82
Post	107 ± 9	367 ± 36*'†’‡	468 ± 6‡
p	P = 0.27	P = 0.00	P = 0.66
Insulin (µIU/ml)			
Pre	2.4 ± 0.5	2.5 ± 0.3	2.4 ± 0.4
Week 3	2.2 ± 0.7	2.6 ± 0.6‡	2.3 ± 0.6
p	P = 0.31	P = 0.57	P = 0.63
Post	2.0 ± 0.3†	2.8 ± 0.3*'†’‡	2.3 ± 0.3
p	P = 0.07	P = 0.04	P = 0.53
HOMA-IR			
Pre	0.3 ± 0.08	2.3 ± 0.1‡	1.8 ± 0.3‡
Week 3	0.4 ± 0.7	1.7 ± 0.2*’⁑’†’‡	2.6 ± 0.3*’‡
p	P = 0.13	P = 0.04	P = 0.03
Post	0.6 ± 0.04*	1.1 ± 0.1*’†’‡	2.7 ± 0.1*’‡
p	P = 0.01	P = 0.00	P = 0.00

Data are presented as means ± SD
* Significant difference compared to pre-test (P ≤ 0.05), ⁑ Significant difference compared to post-test (P≤0.05), † Significant difference compared to CD (P≤0.05), ‡ Significant difference compared to ND (P ≤ 0.05).

for this variable. Both TD and CD groups exhibited significant changes in HOMA-IR levels from pre-training to week 3, and from week 3 to post-test (Table 1). The magnitude of change in this factor from week 3 to post-test was significantly greater in the TD group compared to the CD group (P = 0.01).

As depicted in Figure 2, Catalase activation was significantly lower in diabetic rats than in the ND group (P = 0.00). Aerobic training increased Catalase activation in diabetic rats, with no significant between-group differences. Additionally, Caspase-9 levels were significantly higher in both diabetic groups compared to ND rats (P = 0.00 for both). Furthermore, Caspase-9 levels in response to TD were significantly lower than those in the CD group (P = 0.00).

Cardiac Functional Changes

Echocardiography results indicated that left ventricular end-diastolic volume (LVEDV) significantly decreased in the TD group (0.394 ± 0.12 ml) compared to the CD group (0.431 ± 0.15 ml, P = 0.02) over time. Additionally, this change was significantly lower in the ND group (0.388 ± 0.08 ml) compared to the CD group (P = 0.02).

Regarding left ventricular end-systolic volume (LVESV), findings showed a significantly greater reduction in the TD group (0.156 ± 0.18 ml) compared to the CD group (0.183 ± 0.16 ml, P = 0.02). The ND group (0.147 ± 0.11 ml) also exhibited significantly greater changes compared to the CD group (P = 0.01). Left ventricular ejection fraction (%) demonstrated significantly greater improvement in the TD group (73.8 ± 1.3%, P = 0.03) and the ND group (75.1 ± 0.8%, P = 0.04) compared to the CD group (61.7 ± 0.9%) over time.

Western Blot

Results of western blotting test indicate that both diabetic groups resulted in significantly greater expression of P53 compared to ND group (*P* = 0.02 and *P* = 0.03 respectively). Also, the decrease in the expression of P53 in TD was significantly greater compared to CD group (*P* = 0.001) Figure 3.

Discussion

This study investigated the potential effects of aerobic training with variable intensities on apoptotic indices in the cardiac tissue of diabetic and obese rats. The findings reveal significant reductions in plasma glucose levels, insulin resistance, and the expression of Caspase-9 and P53 proteins following a 6-week

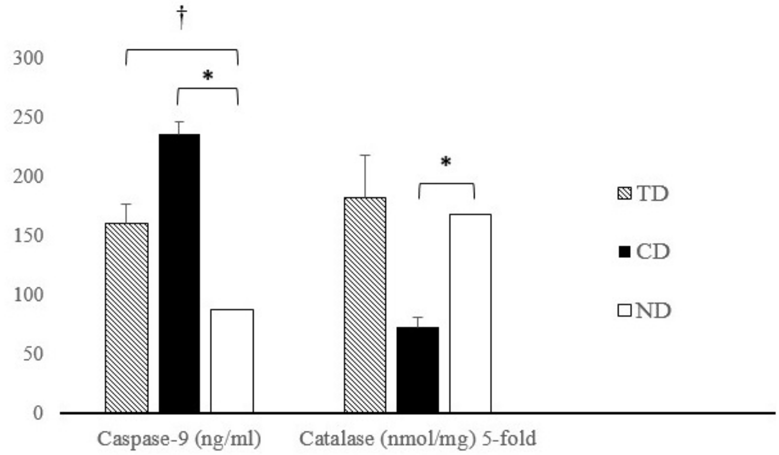

Figure 2. The level of Caspase-9 (nanograms/milliliters) and 5-fold Catalase (nanomole/milligrams) enzymes in diabetic aerobic trained (TD), diabetic control (CD) and non-diabetic healthy rats. † significant difference compared to TD (*P*≤0.05); * significant difference compared to CD (*P*≤0.05)

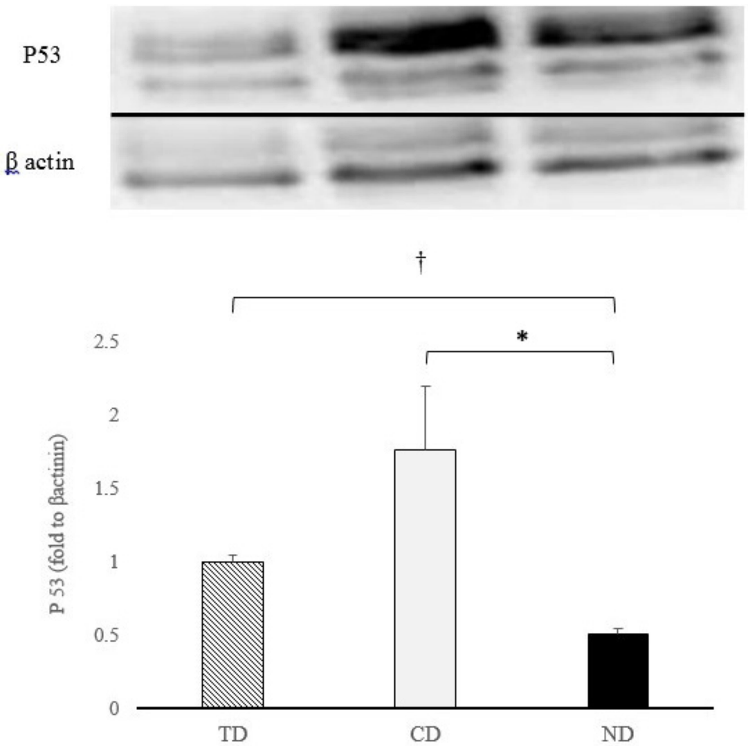

Figure 3. P53 expression (fold changes from β-actinin) in diabetic aerobic trained (TD), diabetic control (CD) and non-diabetic healthy rats. † significant difference compared to TD (*P*≤0.05); * significant difference compared to CD (*P*≤0.05)

aerobic training program with variable intensities. Furthermore, it is well-established that a high-fat diet and STZ-induced diabetes lead to insulin resistance and hyperglycemia.

This study is the first to examine changes in apoptotic indices resulting from aerobic training with variable intensities (ranging from 65% to 85% of running speed at VO_{2max}) in diabetic rats. Consistent with previous research, a negligible reduction in body weight was observed despite improvements in aerobic capacity among male rats[21,35] following aerobic training; the body weight of the trained diabetic rats remained statistically unchanged from pre- to post-training. However, their aerobic capacity was significantly enhanced. Conversely, several studies have shown that consistent endurance training effectively promotes weight loss[36] and improves cardiorespiratory indicators in overweight individuals with type 2 diabetes [26,37].

In line with prior studies that investigated the impact of aerobic exercise training on performance and cardiorespiratory function in healthy individuals[38-40] and obese diabetic subjects[1,41], this study also demonstrated a notable improvement in aerobic capacity after a 6-week training program. Although the aerobic capacity did not significantly change in untrained healthy rats over time, it was markedly lower in untrained diabetic rats compared to the healthy group. The decline in cardiac function due to diabetes seems to contribute to the reduced endurance capacity observed in untrained diabetic rats compared to their healthy counterparts.

Despite hyperglycemia in both diabetic groups, trained diabetic rats exhibited significantly lower blood glucose levels and insulin resistance than untrained diabetic rats after 6 weeks of training. Additionally, insulin resistance decreased significantly after 3 weeks of training, suggesting that 3 weeks of training can initiate protective mechanisms against insulin resistance, but 6 weeks of training is more effective. This corroborates the findings of Lavie et al. (2014) and Pieri et al. (2014), which reported that aerobic endurance exercise improves glucose and insulin metabolism, upregulates Glucose Transporter-4 (GLUT-4) expression—a critical intracellular protein for glucose uptake and utilization—and reduces cardiovascular disease risks in diabetic patients[16,23,42,43].

It is widely recognized that moderate endurance training regulates blood glucose in patients with type 2 diabetes by compensating for impaired energy metabolism in an insulin-deficient state (which protects pancreatic β-cells by increasing insulin-sensitive adenosine monophosphate-activated protein kinase [AMPK] expression), enhancing insulin-mediated glucose transport by increasing protein kinase C expression, promoting insulin secretion and activation, and improving insulin signaling pathway and its downstream protein expressions in the myocardium to ultimately protect cardiomyocytes[43,44].

In accordance with studies by Kwak et al. (2006), Peterson et al. (2008), and Cheng et al. (2013), the current endurance training regimen led to a significant decrease in Caspase-9 and P53. However, the activation of Catalase did not differ significantly between trained and untrained diabetic groups following the training protocol[44]. While French et al. (2008) reported insignificant changes in Catalase in the left ventricle after endurance training, aligning with our findings[9], this contrasts with reports by Kanter et al. (2017) and Naderi et al. (2015), who observed increases in the activity of antioxidant enzymes such as Catalase and reductions in oxidative stress in the hearts of streptozotocin-induced diabetic rats following endurance training[1,11]. The discrepancy may be due to differences in training protocols (voluntary low-intensity aerobic training vs. incremental forced aerobic training), suggesting that aerobic training plays a crucial role in stimulating antioxidant defense through the activation of anti-apoptotic antioxidants. Analogous to these protective changes, and in line with reports from Peterson et al (2009), present study revealed decreases in rate of cardiomyocytes apoptosis following endurance training by decreasing Caspase-9[40]. Instead, Ho et al (2012) reported apoptotic effects of endurance exercise evidenced by increased Caspase-9[43]. The differences in training protocol and the age of participants might be the reason of this controversy. It seems that aging is a potential cause of apoptosis which may negatively triggers apoptotic factors following endurance exercise.

As increasing evidence has demonstrated the role of Caspase-9 on apoptosis, this factor notably triggers the apoptotic pathways following Bax/Bcl-2 ratio regulation immediately after activation in cardiomyocytes[12,16]. Hyperglycemia can promote this activation by cytochrome C release to cytoplasm and triggering cascade activation of caspase-3, which lead to apoptosis of cardiomyocytes. Releasing cytochrome C together with Bax, FAS and P53 into the cytosol forms a complex serving as a platform for Caspase-9 activation as the downstream factor of apoptosis[15]. Therefore, Long-term hyperglycemia in diabetic patients is a strong potent to induce heart failure via increases in Caspase in diabetic patients[6]. The mentioned changes ultimately lead to cardiac function deterioration[3,14]. We assume that such a decrease in this apoptotic factor (Caspase-9) following aerobic training alleviates the vulnerable effects of hyperglycemia on cardiac tissue.

In agreement with previous studies by Cai & Kang (2003) and Gu et al. (2018), Western blot analysis showed a significant increase in P53 protein expression in both diabetic groups, while aerobic training reduced this apoptotic factor in trained rats compared to untrained ones[20,43]. As suggested by Gu et al. (2018), the prevention of functional and pathological heart anomalies in diabetic mice can be achieved through P53 inhibition. This suppression of P53 hinders early apoptotic cell death and prevents subsequent pathogenic effects, including increased cell senescence, impaired glycolysis, and reduced angiogenesis[20].

Schwartzenberg et al. (2004) reported that the upregulation of P53 in diabetic rats may be linked to its role in impeding glucose import via the pentose phosphate pathway and disrupting glycolysis by altering the balance between glycolysis and oxidative phosphorylation. This could account for the observed increase in P53 in diabetic rats and its reduction in trained diabetic rats compared to untrained ones[45].

Furthermore, due to P53's inhibitory effects on the transcriptional activity of GLUT4 (the primary tissue-specific and insulin-sensitive glucose transporter), the suppression of P53 following endurance training may explain the lower insulin resistance index observed in trained diabetic rats compared to untrained diabetic rats.

According to findings related to cardiac function, significant decreases in LVEDV and LVESV, together with significant increases in LVEF approved the improvement in LV function in diabetic rats following aerobic training. These findings are in line with Rodriguez et al (2018) who reported cardiac functional increases in diabetic rats pertained to aerobic training[46]. But, reports from Gharaat et al (2019) which showed significant increase in LVEDV and LVESV after aerobic training is not in agreement with present findings[21]. The difference of cardiac structure following diabetes might be the reason of this contrary. It seems that aerobic training empowers cardiac tissue and promote the function of heart with increasing the ability of contractility due to more EF % in TD.

These observations support the hypothesis that aerobic training programs are as effective as non-pharmacological interventions for improving cardiac function, reducing apoptotic indices, and enhancing antioxidant capacity in diabetic individuals. The current results indicate that incorporating aerobic training into the regimen of diabetic rats may improve fundamental aerobic fitness, boost antioxidant defense mechanisms, thereby reducing cardiac tissue apoptosis, and diminishing the detrimental effects of diabetes on the heart.

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Conflict of Interest

The authors declare that they have no financial associations or conflicts of interest pertaining to the material discussed in the manuscript.

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Author's Contributions

MAG and HC designed the study and performed the experiments and collected data; MS contributed to the data analysis. HC done the literature search. MAG, HC and MS carefully read and approved the final version of the manuscript. MAG edited the final version of the manuscript. MAG and MS re-edited the revised version of the manuscript and prepared it for submission.

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