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A Comparative Analysis of Two High-Intensity Interval Training (HIIT) Programs on PGC-1α, p53, and Citrate Synthase Protein Levels in Cardiomyocytes of Male Type 2 Diabetic Rats

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A B S T R A C T

Introduction: This study investigates the impact of two high-intensity interval training (HIIT) programs on PGC-1α, p53, and citrate synthase (CS) proteins within cardiomyocytes of male type 2 diabetic rats, aiming to discern potential molecular mechanisms influencing cardiac health.

Material & Methods: Twenty-four male Wistar rats were randomly assigned to control (NC), diabetic control (DC), diabetic with type 1 HIIT (HIIT-1), and diabetic with type 2 HIIT (HIIT-2) groups. Streptozotocin (STZ) induced type 2 diabetes, excluding the NC group. A four-week HIIT intervention, six sessions per week, preceded the analysis of heart tissue for PGC-1α, p53, and CS protein levels. Statistical analysis employed GraphPad Prism software version 8 and one-way ANOVA ($P < 0.05$).

Results: Both HIIT-1 ($p=0.004$) and HIIT-2 ($p=0.007$) groups exhibited significantly elevated cardiac PGC-1α levels compared to DC. CS levels increased notably in HIIT-1 ($p=0.001$) and HIIT-2 ($p<0.001$), with HIIT-2 surpassing HIIT-1 significantly ($p=0.010$). Concurrently, p53 levels significantly decreased in both HIIT-1 ($p=0.005$) and HIIT-2 ($p=0.001$) groups compared to DC.

Conclusion: Exercise training (HIIT) positively influences cardiac metabolism, evident in PGC-1α and CS upregulation and p53 downregulation. While these findings provide valuable insights, further exploration is crucial for a comprehensive understanding of the underlying molecular mechanisms. This study advances our understanding of optimizing exercise interventions for improved cardiac health in type 2 diabetes.

Keywords: Exercise Therapy, Type 2 Diabetes Mellitus, Mitochondrial Diseases, PGC-1alpha, Tumor Suppressor Protein p53, Citrate (si)-Synthase

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Introduction

Type 2 diabetes mellitus (T2DM) is acknowledged as a heterogeneous condition arising from the interplay between non-modifiable genetic factors and modifiable environmental influences (1). Traditionally labeled as non-insulin dependent diabetes or adult-onset diabetes, T2DM is characterized by insulin resistance, which may progressively evolve into absolute resistance. However, in the past decade, reduced β-cell function has emerged as a pivotal issue in T2DM (2). Deviations in energy metabolism, including heightened fatty acid metabolism and diminished glucose metabolism, have been documented in individuals with diabetes, leading to a reduction in overall energy production (3). The accumulation of free fatty acids (FFA) in the cardiac tissue and the concomitant decrease in insulin-mediated glucose uptake among diabetic patients contribute to elevated cardiac oxygen consumption and mitochondrial dysfunction. These factors, in turn, precipitate cardiomyocyte death and result in ventricular dysfunction (4, 5).

Emerging evidence suggests that diabetes adversely affects mitochondrial function in the heart, thereby contributing to the development of diabetic cardiomyopathy (DCM) (6). Mitochondrial dysfunction and a reduction in mitochondrial biogenesis have been documented in diabetic patients, with researchers proposing that mitochondrial dysfunction may be implicated as a causative factor in insulin resistance (7). Among the key regulators of the mitochondrial biogenesis process and the expression of genes involved in oxidative phosphorylation is peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) (8). PGC-1α, belonging to a small family of transcriptional coactivators, functions as a metabolic sensor that enables the body to respond to various stimuli such as exercise, fasting, and changes in metabolic substrate availability. Its dysregulation in the context of heart failure has garnered considerable attention (9). Existing evidence indicates that the induction of T2DM in rats through a high-fat diet combined with streptozotocin (STZ) injection is associated with a significant reduction in PGC-1α levels in heart tissue. This reduction, in turn, leads to impaired cardiac mitochondrial function and biogenesis (10).

In spite of the crucial role played by PGC-1α in the promotion of mitochondrial biogenesis, p53 assumes a significant function in the downregulation of PGC-1α and the induction of cardiomyocyte necrosis (11, 12). Acknowledged as a prominent tumor suppressor, p53 emerges as a pivotal contributor to heart failure development (13), exerting regulatory control over diverse cellular functions. Elevated p53 expression fosters apoptosis and holds regulatory significance in cardiovascular health and disease (14). Within the nucleus, active p53 binds to the promoter region, thereby transactivating numerous target genes associated with cell cycle progression, apoptosis, and metabolism (12, 15). Investigations have delineated an augmented expression of p53 in type 2 diabetic rat cardiomyocytes compared to their healthy counterparts, and this heightened p53 expression correlates

significantly with a reduction in PGC-1α and citrate synthase (CS) expression (16). CS, a pivotal regulatory enzyme in the energy-generating metabolic pathway, catalyzes the condensation of oxaloacetate and acetyl coenzyme A to generate citrate in the tricarboxylic acid cycle. It serves as a metabolic marker for assessing oxidative and respiratory capacity (17). Studies have reported diminished CS activity and protein levels of subunits from complexes I and III of the respiratory chain in obese insulin-resistant subjects compared to healthy subjects, underscoring the dysregulation of CS as a contributory factor to insulin resistance and the pathogenesis of diabetes (18).

A sedentary lifestyle, known as the cause of various diseases such as cancer and diabetes, underscores the importance of regular physical activity in promoting overall health and preventing these conditions (19-22). The positive effects of exercise training in preventing cardiac injury and enhancing mitochondrial biogenesis in advanced diabetic cardiomyopathy are linked to the activation of PGC-1α and Akt (23). Despite the favorable impact of various forms of exercise training on diabetes management, a previous study indicated that High-Intensity Interval Training (HIIT) produced comparable, if not superior, improvements in body composition, physical fitness, and glycemic control when compared to continuous endurance training (22, 24). Consequently, HIIT is posited as a time-efficient therapeutic approach for individuals with Type 2 Diabetes (24-26). However, the underlying mechanisms of HIIT effectiveness remain substantially unknown. The current study is undertaken to compare the impact of two types of HIIT programs on the protein levels of PGC-1α, p53, and citrate synthase in the cardiomyocytes of male Type 2 diabetic rats.

Materials and methods

Animal

This semi experimental study, conducted on the 24 male Wistar rat (age ranging 5 o 6 weeks, 280 to 350 g weight) purchase from Razi Pasteur institutes. The rats were kept under a 12 h light–dark cycle (temperature: 22±3 C°) in tarbiat modares university animal house in similar environment and condition. The rats randomly assigned in four equal groups (6 rat in each group) including normal healthy control (NC), diabetic control (DC), type1 (HIIT-1) and type2 (HIIT-2) HIIT protocol. The animal in all groups had ad libitum access to chow and water throughout intervention period.

Inducing type 2 diabetes (T2DM)

T2DM induced in the rats in all groups except NC group. For this purpose, following 12 hours night fasting the nicotinamide solution (110 mg/kg) were injected intraperitoneally. After 15 minutes, streptozotocin (STZ) freshly dissolved in (pH 4.5) citrate buffer injected intraperitoneally (60 mg.kg), dissolved citrate (0.05 mol) was gavage (12). The 72 hours after nicotinamide+STZ injection and in order to T2DM confirmation, the blood was collected from the tip of the tail vein for measurement of fasting blood glucose by glucometer, and blood glucose more than 200 mg/dl considered as T2DM. The rats

body weight and fasting glucose levels in the different group reported in table1

Table 1. Body weight changes and glucose level in different group (mean±SD).

Variable	NC	DC	HIIT-1	HIIT-2
Baseline weight (g)	318.3±22.7	322.7±11.3	328.7±18.3	321.2±18.0
Final weight (g)	376.2±33.8	282.5±39.3	268.7±42.5	301.1±55.3
Glucose/BG (mg/dl)	185.5±12.7	567.3±62.5	491.5±33.4	427.5±53.1

NC: Healthy control, **DC:** Diabetic control, **HIIT:** High intensity interval training, **BG:** Blood Glucose.

High intensity interval training (HIIT) program

Rats were familiarized with treadmills before exercise training began (10-15 min per day) with the intensity of 6 m/min for one week. Firstly, aerobic capacity of animals determined as previously reported procedure (27). The conducted exercise training program including two types of HIIT, both of them consist of three min warm up and three min cooldown with 40 % and 30% of rat maximum speed respectively. The type 1 HIIT protocol (HIIT-1) consist of 4 or 5 intensive intervals in the two first (1-2) and two final (3-4) weeks of training program respectively. High intensity intervals in the first week, second week and 3-4 weeks conducted with 80%, 85% and 90% of determined rat maximum speed respectively. Each intensive interval duration was 2 min followed by 2 min low intensity interval with 40% of maximum speed. The type 2 HIIT protocol (HIIT-2) conducted with 5 intensive intervals in the first two weeks and 7 intervals in the two final weeks. Each intensive interval duration was 2 min followed by 2 min low intensity interval with 30% of maximum speed. The intensity of HIIT-2 protocol was similar to HIIT-1 protocol and covering distance of both HIIT protocol was similar. The both training program Properties have been reported in the table 2.

Table 2. Four weeks of HIIT program

Variable	Training	First week	Second week	Third week	Fourth week
Maximum speed when reaching VO2max (m/min)	HIIT-1	15	18	20	20
	HIIT-2	12	6	18	18
Training session time (min)	HIIT-1	6	8	10	12
	HIIT-2	6	8	10	12
Intensity (m/min)	HIIT-1	12	16	18	18
	HIIT-2	9	10	10	12

Blood sampling and tissue retrieval

After a 48-hour fasting period following the conclusion of the final training session, animals were subjected to anesthesia using xylazine (10 mg/kg body weight, I.P.) and ketamine (90 mg/kg body weight, I.P.). Heparin-containing tubes were employed to collect blood samples from the hearts. After centrifugation at 15 °C and 3000 rpm for 15 minutes, serum was isolated and preserved at -80 °C for subsequent analysis. left ventricle were promptly harvested, flash-frozen in liquid nitrogen, and stored at -80 °C for future experimentation.

Blood glucose assessment

For the determination of fasting blood Glucose (BG), the glucose oxidase technique was employed, employing a quantitative glucose assay kit (Pars Azmoon, Karaj, Iran).

Western blotting

Western blot analysis was conducted on left ventricle cellular proteins, with 70-100 mg of tissue homogenized in RIPA buffer (pH 7.4, 1% Triton X-100, 50 mM Tris–HCl, 0.2% SDS, 0.2% sodium deoxycholate, 1 mM Na-EDTA, and 1 mM PMSF). The protein extracts, treated with PMSF and a protease inhibitor cocktail (Roche, Mannheim, Germany), were examined for changes in protein abundance. Total protein concentrations were determined using the Bradford assay. Subsequently, equal protein amounts from each sample were separated via SDS–PAGE, transferred to PVDF membranes, and blocked with 5% non-fat dry milk or bovine serum albumin in tris-buffered saline with 0.5% Tween-20 for two hours at room temperature. Primary antibodies targeting PGC-1α (Cell signaling, Beverly, USA), p53 (SantaCruz, California, USA), CS (Cell signaling, Beverly, USA), and GAPDH (Cell signaling, Beverly, USA) were used for overnight incubation at 4 °C. Visualization of target protein bands occurred with an enhanced chemiluminescent substrate following incubation with second HRP-conjugated antibodies (horseradish peroxidase). Band densities were analyzed using Image-J for densitometry (23).

Statistical analysis

The data obtained in the present study were subjected to analysis using GraphPad Prism software version 8. The normality of the data was assessed using the Shapiro-Wilk test, which indicated that the data followed a normal distribution. Between-group differences were assessed using a one-way ANOVA test, followed by the Tukey post hoc test for pairwise comparisons. Statistical significance was considered at the $P<0.05$ level.

Results

In the present study, all animals (normal and diabetic male Wistar rat) completed considered intervention (healthy or diabetic control, and HIIT program) and included in the final data analysis. One-way ANOVA test represented a significant between group difference for cardiac PGC-1α ($p<0.001$), CS ($p=0.009$) and p53 ($p<0.001$) levels. Therefore, Tukey post hoc test was used for comparing different groups together.

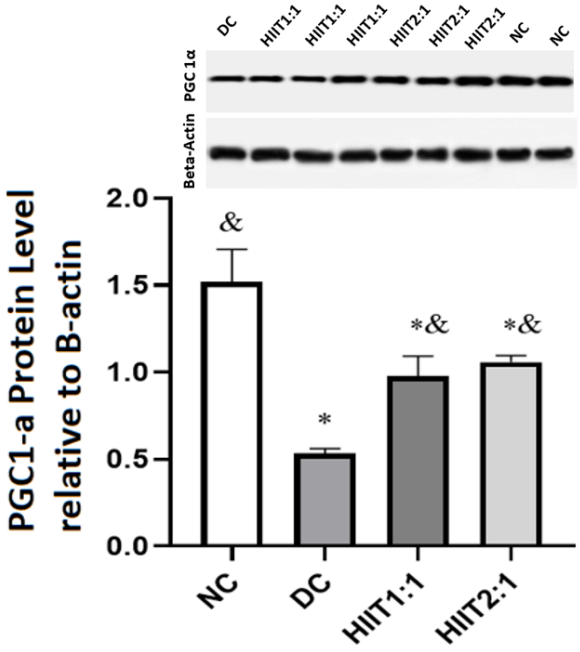

Figure 1. PGC-1α protein levels in cardiomyocyte. * Significant difference with NC group. **&** Significant difference with DC group.

Present study findings indicated that cardiac PGC-1α levels decreased significantly in the DC, HIIT-1 and HIIT-2 groups compared to NC group ($p<0.05$). The levels of PGC-1α in the HIIT-1 ($p=0.004$) and HIIT-2 ($p=0.007$) groups was significantly higher compared to DC group. However, there was no significant difference between two trained groups (HIIT-1 and HIIT-2) for observed changes in the PGC-1α levels ($p=0.585$) (Figure 1).

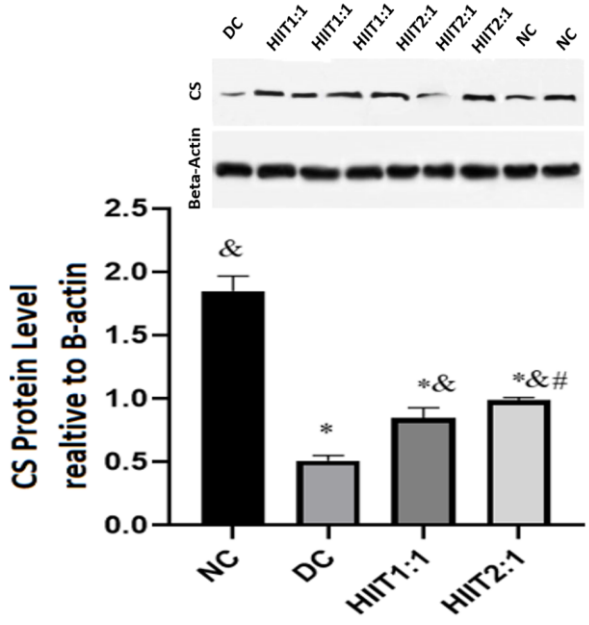

Figure 2. Citrate synthase (CS) protein levels in cardiomyocyte. ⁎ Significant difference with NC group. **&** Significant difference with DC group. **#** Significant difference with HIIT-1 grou

The CS protein levels indicated a significant decrease in the DC, HIIT-1 and HIIT-2 groups compared to NC group ($p<0.05$). The CS protein levels in the HIIT-1 ($p=0.001$) and HIIT-2 ($p<0.001$) groups significantly increased compared to DC group. In addition, significant difference was observed for CS protein between two trained groups (HIIT-1 and HIIT-2) and CS protein level in the HIIT-2 compared to HIIT-1 group was significantly higher ($p=0.010$), emphasized the further effect of HIIT-2 on CS protein (Figure 2).

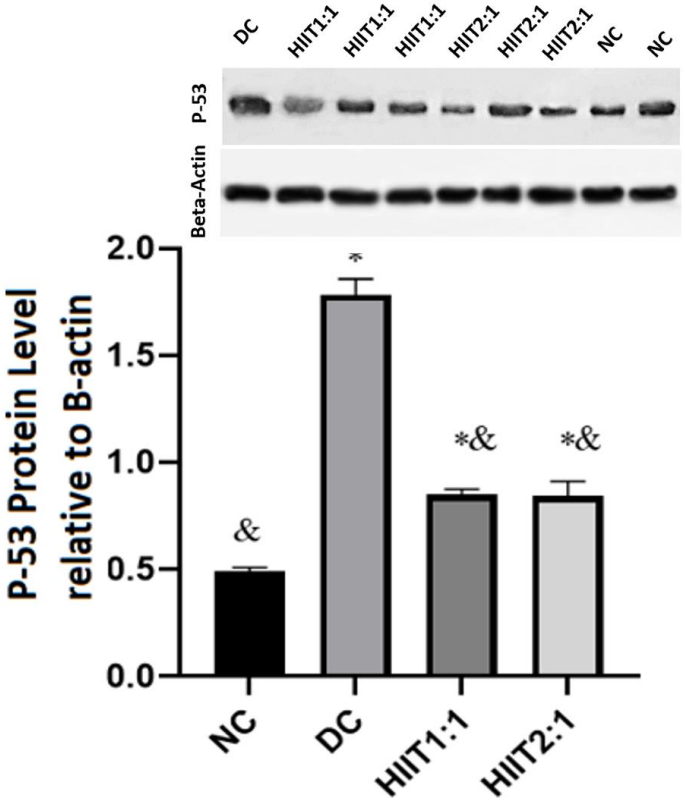

Figure 3. p53 protein levels in cardiomyocyte. * Significant difference with NC group. **&** Significant difference with DC group

Tukey post hoc test indicated a significant increase in cardiac p53 levels in the DC, HIIT-1 and HIIT-2 groups compared to NC group ($p<0.05$). On the other hand, significant decrease in p53 protein levels were observed in the HIIT-1 ($p=0.005$) and HIIT-2 ($p=0.001$) groups compared to DC group. However, there was no significant difference between HIIT-1 and HIIT-2 groups for observed changes in the cardiac p53 levels ($p=0.990$) (Figure 3).

Discussion

The present study main findings were that four weeks HIIT in type 2 diabetic rat result in significant increase in PGC-1α and CS proteins levels in the cardiomyocyte, and there was no significant difference between two exerted HIIT protocol for observed changes in PGC-1α levels, but CS increase in HIIT-2 group was significant compared to HIIT-1 group. Yan et al (2013) reported that impairment of AMPK-PGC-1α signaling

results in mitochondrial biogenesis dysfunction, and this is a novel mechanism responsible for the enhanced susceptibility of diabetic hearts to myocardial infarction (MI) and interventions that restore this signaling axis may have therapeutic potential for treating diabetic heart injury (28). In fact, pharmacological targets and drugs that modulate mitochondrial biogenesis, considered as a therapeutic strategy for the treatment of type 2 diabetes and insulin resistance (29).

Exercise training known as important stimulator for inducing mitochondrial biogenesis by activating different mechanism including PGC-1α pathway (30), which present result supported this statement. Present findings regarding the PGC-1α upregulation following exercise training confirmed in the previous study. wang et al (2020) reported that inducing diabetes with streptozotocin (STZ) injection in the male C57BL/6 mice is associated with decreasing cardiac PGC-1α compared to non-diabetes mellitus sedentary control group (31). In contrast, these researchers reported that eight weeks endurance training on mouse treadmill result in significant increasing in cardiac PGC-1α and AMPK protein expression and concluded that exercise training can ameliorates cardiac dysfunction by decreasing ROS production, enhances the mitochondrial oxidative capacity in the diabetic heart and exercise training shifts energy metabolism from fatty acid oxidation to glucose oxidation (31). In addition, SIRT1/PGC-1α/PI3K/Akt pathway downregulation after MI, restores by post-MI 4-week treadmill exercise training, and similar to MI group, activation of SIRT1/PGC-1α/PI3K/Akt signaling pathway were observed in healthy adult male rats (32).

In addition, some researchers attributed the protective effects of exercise training (three weeks swimming) against acute rodent model myocardial infarction to improving myocardial energy metabolism and mitochondrial biogenesis by an activation of PGC-1α in heart tissue, which cardiac PGC-1α upregulation by swimming training was associated with significant increase in expression of other genes involved in mitochondrial biogenesis such as TFAM, NRF-1 and NRF-2, and simultaneous decrease in apoptotic genes including caspase 3 and BAX were observed (33). Unfortunately, in the present study the changes in the above-mentioned genes don't investigate. However, we observed that cardiac p53 protein as an important apoptosis stimulator decreased in diabetic rat after four weeks HIIT. Studies have reported that p53 and associated apoptosis play important roles in the pathogenesis of ischemia/reperfusion caused acute cardiac diseases (34). p53 promotes cardiac dysfunction in diabetes via excessive mitochondrial respiration-mediated oxidative stress and lipid accumulation (35). On the other hand, inhibition of p53 prevents diabetic cardiomyopathy by preventing early-stage apoptosis and cell senescence (36), and according to our findings high intensity interval training is effective strategy for p53 downregulation. Al-Jarrah et al (2012) supported our findings and suggested that levels of p53 increased in the heart of diabetic rats, and 4-week

treadmill training program significantly decreased the expression level of p53 in in the cardiac muscle (37). Moreover, decreased in p53 protein levels have been observed after 8 weeks endurance training on treadmill in other tissue including skeletal muscle of type 2 diabetic Goto-Kakizaki rats, which result in attenuates oxidative stress and researchers concluded that p53 downregulation is important mechanism for exercise-induced increase in mitochondrial content and function (38). Since, elevated p53 levels correlate with cardiomyocyte apoptosis and impairment of mitochondrial function (39), its inhibition plays an important role for trigger the exercise training induced adaption in the heart tissue. Although, the changes in oxidative stress markers and mitochondrial content and function in our research is not studied, but despite observed decrease in the p53 levels and simultaneous increase in CS and PGC-1α levels as mitochondrial biogenesis stimulators, it can be concluded that mitochondrial biogenesis probably increased in the present study.

In corroborate with our findings regarding CS upregulation in cardiac tissue by exercise training, Siu et al (2003) reported that eight weeks aerobic training in Sprague-Dawley rats cause to significant enhancement of expression and enzyme activity of citrate synthase in cardiac and skeletal (soleus) muscles (40). Moreover, increase in CS activity in untrained and trained human skeletal muscle have been reported, although the mechanism of this increase is unknown (41). Some researchers observed enhancing CS activity following different type of exercise training including HIIT and showed a positive and significant linear relationship between CS activity and VO2max (42). Collectively, present study findings suggested that HIIT protocol may result in improving cardiac metabolism through CS and PGC-1α upregulation as a stimulator of mitochondrial biogenesis and decreasing p53 level which play important role in cardiomyocyte apoptosis and impairment of mitochondrial function.

Conclusion

In conclusion, this study sheds light on the positive impact of four weeks of HIIT on cardiac tissue in type 2 diabetic rats. The observed increases in PGC-1α and CS protein levels, coupled with a decrease in p53, suggest potential mechanisms contributing to improved mitochondrial function and biogenesis. These findings align with the broader literature on exercise-induced adaptations and hold promise for addressing cardiac dysfunction in diabetes. However, several limitations, including the focused protein-level analysis, the absence of exploration into broader molecular markers, and the reliance on a rat model, should be acknowledged. While our results provide valuable insights, further research is warranted to delve into the long-term effects, individual-specific responses, and broader molecular changes associated with HIIT. Understanding these aspects will not only refine our comprehension of the mechanisms at play but also enhance the translatability of these findings to human populations. In essence, the current study sets the stage for future investigations to unravel the full spectrum of benefits and

considerations associated with incorporating HIIT as a potential therapeutic strategy for mitigating cardiac complications in type 2 diabetes.

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Conflict of interest

The authors declare that no conflict of interest exists.

Authors' contributions

N Kh. conducted the study protocol, M. P. designed and confirmed the training protocol, and M A A. analyzed the research data. The article draft was prepared collaboratively by N Kh. and M A A., and the final manuscript was edited by M P. All authors contributed equally to data collection.

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