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Original Research

Acute exercise induces comparable mitochondrial biogenesis and vascular angiogenesis molecular regulators in the diabetic and non-diabetic rat myocardium

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Abstract

Background: The diabetic heart is associated with mitochondrial and microvascular dysfunctions along with impaired downstream co-activators. These functions may improve with long-term exercise training. However, it is unknown how they respond to acute exercise in the diabetic compared to the non-diabetic heart.

Objective: Therefore, we aimed to evaluate and compare the acute molecular responses of mitochondrial biogenesis and angiogenic and anti-angiogenic factors to a single exercise session between the diabetic and non-diabetic heart.

Methods: Male Sprague-Dawley rats were assigned into one of four groups: 1- non-diabetic sedentary control (CS); 2- Diabetic exercise (DIEX); 3- Diabetic sedentary (DIS); and 4- non-diabetic exercise (CEX) groups. Left ventricles were obtained 6 – 7 weeks following diabetes induction and following a 60-min of treadmill running from CEX and DIEX.

Results: Vascular endothelial growth factor and hypoxia-inducible factor 1α mRNA was not different among all groups. Thrombospondin-1 mRNA was not different in CEX and DIEX and was greater in both groups than CS and DIS ($P < 0.005$). Peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC-1α) expression was not different in CEX and DIEX ($P = 0.4$). PGC-1α expression was lower in CS compared to all groups ($P < 0.001$ for CEX and DIEX, and $P = 0.01$ for DIS *vs.* CS) (Malondialdehyde was not different in all groups.

Conclusion: All the above molecular responses to acute exercise were similar in the diabetic and non-diabetic hearts. This may suggest that the diabetic heart may similarly benefit from exercise in terms of mitochondrial biogenic and vascular angiogenic functions as the non-diabetic heart. Future studies should investigate the long-term training responses.

Keywords: *Cardiomyopathy; Endurance training; Oxidative stress; PGC-1α; Thrombospondin*

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1. Introduction

Diabetic cardiomyopathy is a primary complication of diabetes mellitus, characterized by increased risk of heart failure among diabetic patients, independent of other risk factors such as hypertension, coronary artery disease, obesity, dyslipidemia, and age (1). Furthermore, diabetes is an independent predictor of morbidity and mortality of patients with heart failure and left ventricular dysfunction (2). Thus, urgent therapeutic interventions are imperative to mitigate the multifaceted complications associated with diabetes.

The pathogenesis of diabetic cardiomyopathy develops over a prolonged periods of abnormal control of glycaemia, and systemic and cardiac insulin resistance (3). Impaired

glycemic control reduces cardiac efficiency and contractility and exacerbates catecholamine-mediated myocardial injury (4, 5). These diabetic risk factors contribute to the development of diabetic cardiomyopathy via several mechanisms, including impaired cardiac blood flow, and mitochondrial and microvascular dysfunction (3-5).

Peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α is a central transcriptional coactivator, regulating numerous mitochondrial functions, including mitochondrial biogenesis, oxidative phosphorylation, and the ratio of fatty acids to glucose oxidation (6, 7). The cellular content of PGC-1α has been shown to increase via different stimuli such as cold exposure, fasting, exercise (8), and more recently following postexercise cold exposure (9, 10).

Diabetic skeletal muscles exhibit a reduced PGC-1α gene expression associated with reduced oxidative phosphorylation (11). In contrast, the diabetic myocardium exhibits increased PGC-1α expression in association with increased mitochondrial biogenesis and fatty acid oxidation, but with reduced mitochondrial energetic efficiency (12). Despite the increased mitochondrial density in the diabetic hearts with increased PGC-1α, mitochondrial oxidative stress, and uncoupling protein activity were elevated and associated with reduced cardiac efficiency (13). However, other studies reported reduced PGC-1α mRNA in the diabetic heart at later stages of diabetes (14), suggesting the complexity of these molecular mechanisms in the diabetic cardiomyopathy.

Diabetes is associated with 2 – 4 folds increases in myocardial microvascular and macrovascular complications among diabetic compared to non-diabetic patients (15). It has been suggested that hypoxia-induced collateral coronary neovascularization is impaired in the diabetic heart by the effect on endothelial function (16). Vascular endothelial growth factor (VEGF) is a major angiogenic mediator in the myocardium that plays a pivotal role in the process of coronary collateral vessels formation and regulation, induced by hypoxia and hyperglycemia (17). Moreover, during the course of diabetic cardiomyopathy, reduced VEGF mRNA and protein expression was prior to the onset of cardiomyopathy impairments, highlighting the potential role of VEGF in its development. The expression of VEGF is primarily regulated by the transcription factor hypoxia-inducible factor-1α (HIF-1α) in response to hypoxia, which primarily regulates the dimerization of HIF-1α and HIF-1β to form HIF-1 (18).

During exercise, there is a multi-folded increase in cardiac metabolic demands. Chronic exposure to exercise training induces heart structural and molecular remodeling, characterized by elevated oxidative energy production capacity, improved mitochondrial biogenesis and efficiency, and increased cardiac capillarization (19). PGC-1α and VEGF, along with their upstream and downstream mediators, are though as primary regulators in these adaptations (9, 19, 20). These long-term adaptations are thought to arise from the cumulative effects of each single exercise session. Exercise induces perturbations in homeostasis during and following exercise, triggering signal transduction pathways and downstream molecular transcriptions, coupled with effector proteins translation. These molecular pathways, when regularly repeated, accumulate to promote chronic changes in the abundance, regulation, and maximal activity of effector proteins that are involved in cellular bioenergetics, remodeling, and organelle biogenesis—underpinning chronic adaptations to exercise training (21). In particular, chronic enhancement of mitochondrial biogenesis and efficiency and angiogenic function may result from the cumulative effects of transient increased transcription of PGC-1α and VEGF mRNA following each exercise session (22). Therefore, identifying the acute myocardial molecular responses to exercise may provide insights into the longer term cardiac mitochondrial and vascular adaptations. Nonetheless, while data exist on the acute effect of exercise on PGC-1α, VEGF, and HIF-1 mRNA expression in the skeletal muscles (23), studies investigating these molecular responses to acute exercise in the myocardium, especially in the diabetic heart, are limited. We have previously shown increased myocardial PGC-1α protein and VEGF mRNA expression following 10-week running exercise training in non-diabetic rats (9, 10). However, the acute effect of exercise on myocardial content of these coactivators mRNA still unexplored. Moreover, the differences in these molecular responses between diabetic and non-diabetic hearts are largely unknown.

Therefore, this study aimed to investigate the acute responses of mitochondrial biogenic regulator, PGC-1α, and the angiogenic factors, VEGF and HIF-1α, along with anti-angiogenic gactor thrombospondin-1 (TSP-1) mRNA to a single session of exercise in the diabetic and nondiabetic myocardium. In addition, we aimed to examine any differential responses of these molecular factors and oxidative stress between the diabetic and non-diabetic myocardium. We hypothesized that the diabetic heart would exhibit attenuated PGC-1α and VEGF molecular responses,

along with elevated oxidative stress, following an exercise session compared to the non-diabetic heart.

2. Material and methods

2.1. Animals and experimental design

Forty-three adult male Sprague-Dawley rats were obtained from the animal house of the Department of Biological Sciences, Yarmouk University, Jordan. The rats were housed in cages of 3 – 4 rats in a controlled environment with a 12:12 h light-dark cycle at 23 – 24 °C, 55% humidity, and provided with rat chow and water ad libitum. Rats were assigned into one of four groups: 1- non-diabetic sedentary control (CS); 2- Diabetic exercise (DIEX); 3- Diabetic sedentary (DIS); and 4- non-diabetic exercise (CEX) groups. To account for the likelihood of higher mortality and unsuccessful diabetes induction, more rats were intentionally allocated to the diabetic groups. Throughout the experimental period, four rats died and three did not develop diabetes from the diabetic groups. In addition, one rat died from the non-diabetic groups. Consequently, only 35 rats were included in the analysis and assigned as follows: (CS; n 8); (DIEX; n 9); (DIS; n 9); and (CEX; n 9). Accordingly, a post hoc power analysis was performed with the remaining total animals (n = 35), assuming effect size of 1 and $\alpha = 0.05$. The analysis resulted in a statistical power of 0.99. The animals aged 10 – 12 weeks old with initial body weight of 200± 50g. All procedures were approved by local committee at Yarmouk University, Jordan following the Guide for the Care and Use of Laboratory Animals (IACUC/2020/2).

2.2. Diabetes induction

Diabetes was induced in both groups DIEX and DIS using streptozotocin (STZ) injection as described previously (24). Animals received a single intraperitoneal STZ injection (Sigma-Aldrich, USA) (55 mg/kg dissolved in 0.9 % normal saline) after an overnight fast. Rats were provided with 10% sucrose ad libitum water for 24 h after STZ injection to avoid hypoglycemia. Subsequently, their diet was switched back to normal chow and water ad libitum. Diabetes was confirmed if blood glucose was > 250 mg/dl 48h after STZ injection. Rats failing the diabetes criterion were injected with another dose of STZ. following the second STZ injection, all animals with fasting blood glucose less than 250 mg/dl were excluded from the study. The confirmed diabetic rats remained in their cages for another 6 – 7 weeks. This duration aligns with the timeframe (4 – 6 weeks) required for the development of diabetic cardiomyopathy in Sprague-Dawley rats following similar STZ dose (i.e. 55 mg/kg). Diabetic cardiomyopathy was indicated in these models by left ventricular remodeling and diastolic dysfunction (25, 26).

2.3. Acute exercise protocol

DIEX and CEX rats were habituated for 5 consecutive days to a motorized treadmill running (Panlab Harvard Apparatus, model LE8710MTS, Barcelona, Spain). Running speed was progressively increased, ranging between 28 to 50 m/min for 5 – 50 min. The animals were allowed for 72 h of complete rest after the last habituation session to eliminate any remaining effect of exercise. DIEX and CEX only rats performed a single bout of treadmill running at 50 m/min, 0% grade for 60 min at 23 – 24 °C, 55% humidity. This exercise intensity (speed and grade) was selected to ensure the animals exercised at or above their 65% maximal oxygen uptake according to previous data using the same strain (27). Given the short period of familiarization, no incline was applied grade during running to reduce the risk of injuries related to electrical grid contact. All over, the selected intensity and duration were previously shown to induce acute and long-term responses at the molecular, protein, and functional levels in the myocardium of rats (24, 27, 28). Thus, this protocol provides an adequate exercise stimulus to investigate the acute molecular response in both diabetic and non-diabetic hearts.

2.4. Tissue removal and preparation

As described previously (24), the rats were decapitated after 1.5 – 2 h of the acute exercise session. The hearts were quickly removed, rinsed by Phosphate-buffered saline (PBS 1x), weighed, and dissected to separate the right and left ventricles. Left ventricle samples were immediately frozen in liquid nitrogen and stored at -80°C.

2.5. RNA isolation and quantification

Left ventricular tissue (~ 25 mg) was homogenized in 600 µl of trizole reagent in bead mill homogenizer (Bead ruptor 4, OMNI, US). Total RNA extraction from the homogenate was performed following the procedures of an RNA isolation kit instructions, with additional DNAase treatment and 25 µl elution (Direct-Zol™ RNA MiniPrep, CA, USA). The extracted RNA was validated using 1.5% denaturing

agarose electrophoresis and NanoDrop spectrophotometer technology (NanoDrop™ 2000, Thermo Scientific) for integrity and purity, respectively. Integral RNA was confirmed by visualizing undegraded 28S and 18S ribosomal RNA bands at an approximate 2:1 ratio. RNA purity was validated with 260/280 and 260/230 wavelength absorption ratios both around 2 (9). The validated RNA samples were stored at -80°C for further analysis.

Complementary DNA (cDNA) was synthesized by reverse transcription from 500 ng of total RNA using oligo-(dT) primer according to the manufacturer's instructions (Cat. #RR036A, Takara, Japan) and as described previously (24).

Quantitative real time PCR (RT-PCR) was conducted using SYBER Green Premix Ex TaqII™ master mix (Cat. #RR820L, Takara, Japan) on Line-Gene 9600 Real-Time PCR system (Bioer Technology, Bingjiang, China). PGC-1α, VEGF, thrombospondin (TSP-1), HIF-1α, and the control housekeeping gene Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) mRNA were quantified (Table 1). The $2^{-\Delta\Delta CT}$ method was used to analyze the RT-PCR data (29). The change in mRNA expression was calculated by normalizing the threshold cycle (C_T) of the target gene to C_T of the reference gene GAPDH. The untreated CS was used as the calibrator.

Table 1. Primers sequence

Gene	Forward Primer	Reverse Primer
GAPDH	*GCTCTCTGCTCCTCCCTGTTCT*	*TACGGCCAAATCCGTTCACACC*
HIF-1α	*AAGAAAGAGCCCGATGCCCTGAC*	*TCCGTGTCATCGCTGCCGAAGT*
PGC-1α	*ACAGACACCGCACACATCGC*	*TCATAGCTGTCATACCTGGGCCTAC*
TSP-1	*TGTCCGATTGATGGATGCCTGTCC*	*CGCACCACATTTCCAGCTACCA*
VEGF	*AGCAGAAAGCCCATGAAGTGGTG*	*GGAAGATGTCCACCAGGGTCTCA*

GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase; **HIF-1*α*,** Hypoxia-inducible factor-1; **PGC-1*α*,** Peroxisome proliferator-activated receptor (PPAR)-γ coactivator; **TSP-1,** Thrombospondin; **VEGF,** Vascular endothelial growth factor

2.6. Lipid peroxidation

Lipid peroxidation, an indicator of oxidative stress in the myocardium, was measured by the malondialdehyde (MDA) assay. MDA was assayed in the form of thiobarbituric acid (TBA)-MDA adduct following the instructions of a MDA assay kit (ab118970, Abcam) (30). Absorbance was read with a microplate reader at 532 nm (Multiskan™ FC Microplate Photometer, Thermo Scientific).

2.7. Statistical analysis

The Statistical Package for Social Sciences software (SPSS, Chicago, IL, version 20.0) was used to analyze all statistical analysis. Data are presented as mean ± STDV. The analysis of variance (one-way ANOVA) was used to detect the main effects of exercise, diabetes, and combined exercise and diabetes on mRNA gene expressions and the level of lipid peroxidation, followed by the least significance difference method for the pairwise comparisons. Homogeneity of variance was tested using Leven's statistics. Significance was set at $P < 0.05$. Cohen's *d* effect size was calculated to indicate trivial, small, moderate, large, and very large effect when the *d* was < 0.2, 0.2 – 0.6, 0.6 – 1.2, 1.2 – 2.0, and >2.0, respectively (31-33).

3. Results

3.1. Animals Characteristics

Initial and final body weight, heart weight, and heart to body weight ratio for the same set of animals were previously published in our related work ***(***24***)***. To avoid redundancy, these specific results are not reiterated here.

3.2. Acute responses of gene expression – real-time PCR

There was no significant main group effect observed for acute VEGF (1.0 ± 0.25, 1.2 ± 0.4, 1.6 ± 0.9 and 1.6 ± 0.9 folds for CS, DIS, CEX, and DIEX, respectively; $P = 0.3$; ; $d = 0.0 – 0.8$ for all pairs) and HIF-1α mRNA expression (1.01± 0.2, 1.3 ± 0.3, 0.9 ± 0.4, and 1.0 ± 0.3 for CS, DIS, CEX, and DIEX, respectively; $P = 0.08$; $d = 0.04 – 0.34$ for CS, DIEX, and CEX comparisons, and $d = 1.0 – 1.13$ for all comparisons with DIS), with their expression remaining unchanged relative to CS in all groups ($P = 0.1 – 0.8$) (Figure 1).

A significant main group effect was observed for acute TSP-1 mRNA expression ($P < 0.001$). Both CEX and DIEX showed similar levels of TSP-1 mRNA expression (6.8 ± 2.6 and 5.7 ± 3.6 folds, respectively; $P = 0.9$; $d = 0.35$), with higher of TSP-1 mRNA expression compared to CS and DIS

(1.04 ± 0.35 and 1.5 ± 0.6 folds, respectively) ($P < 0.005$ for all comparisons; $d = 1.6$ and 1.8 for DIEX vs. CS and DIS and $d = 2.8$ and 3.1 for CEX vs. CS and DIS). There was no significant difference in TSP-1 mRNA expression between DIS and CS ($P = 0.7$; $d = 0.9$) (Figure 1).

PGC-1α mRNA expression was different among the groups ($P < 0.005$). PGC-1α mRNA expression in CS (1.02 ± 0.2) was significantly lower than CEX, DIEX and DIS (2.6 ± 0.6, 2.3 ± 0.8 and 1.8 ± 0.5 folds; $P < 0.001$ for CEX and DIEX, and $P = 0.01$ for DIS, and $d = 3.5$, 2.2, and 2.05 for CS vs CEX, DIEX, and DIS, respectively). PGC-1α mRNA expression in CEX was higher than DIS but similar to DIEX ($P = 0.009$ and 0.4, and $d = 1.44$ and 0.42 respectively) (Figure 1).

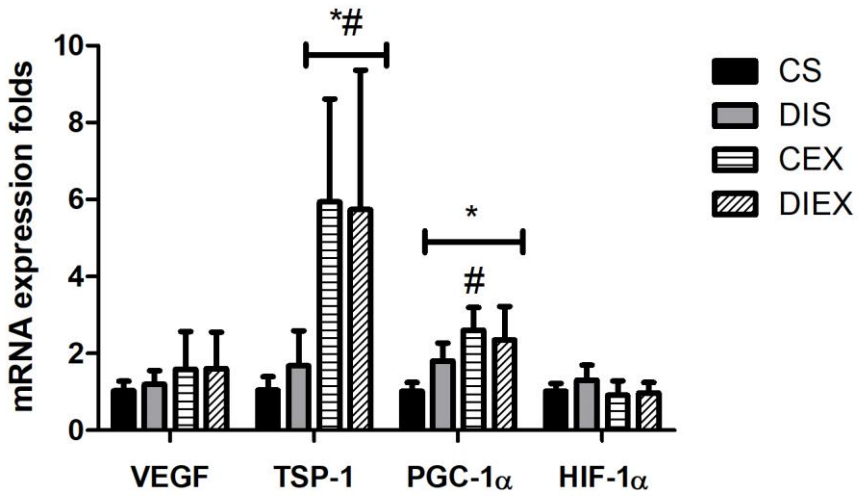

Figure 1. The mRNA expression folds relative to CS of VEGF, TSP-1, PGC-1α, and HIF-1α obtained from RT-PCR.

* Significant difference with CS at P < 0.05.

\# Significant difference with DIS at P < 0.05.

CS, non-diabetic sedentary control; **DIEX,** Diabetic exercise; **DIS,** Diabetic sedentary; **CEX,** Non-diabetic exercise; **HIF-1α,** Hypoxia-inducible factor-1; **PGC-1α,** Peroxisome proliferator-activated receptor (PPAR)-γ coactivator; **TSP-1,** Thrombospondin; **VEGF,** Vascular endothelial growth factor

3.3. MDA

Figure 2 shows the MDA concentration (nmol/mg tissue) for all groups. There was no group main effect observed for the MDA levels ($P = 0.9$; effect size was trivial to small for all comparisons $d = 0.13 – 0.44$).

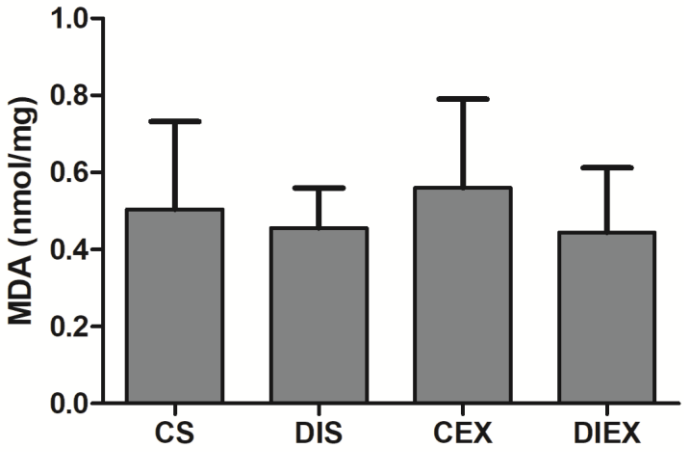

Figure 2. MDA concentration in nmol/mg of the left ventricular tissue for the groups CS, CEX, DIS, and DIEX. MDA was measured using the thiobarbituric acid assay to indicate the level of lipid peroxidation. No main group effect was observed.

CS, non-diabetic sedentary control; **DIEX,** Diabetic exercise; **DIS,** Diabetic sedentary; **CEX,** Non-diabetic exercise; **MDA,** malondialdehyde

4. Discussion

This study aimed to evaluate the differential responses of VEGF, PGC-1α, TSP-1, and HIF-1α mRNA expression to a single bout of exercise in the myocardium of diabetic and nondiabetic rodents. Primarily, our findings showed that a single exercise session induced similar mRNA expression of all the examined genes in the diabetic and non-diabetic left ventricles.

In this exercise protocol, VEGF and HIF-1 mRNA expression was not changed in all groups relative to the control sedentary rats. To date, no studies have investigated the acute effect of exercise on the mitochondrial biogenesis and vascular angiogenic factors in the myocardium, particularly in the context of the diabetic heart. Rather, the expression of these factors was examined following a long term exercise training in the myocardium and following an acute exercise in the skeletal muscles (9, 34, 35). In skeletal muscles, an one hour exercise bout elicited an increase in VEGF mRNA muscles up to 4 h postexercise and returned to baseline by 8 and 24 h postexercise (35). Further investigations revealed that acute exercise effect demonstrates a fiber-type pattern, by which only type IIb fibers exhibited an increased VEGF mRNA expression compared to types I, IIa, and IIx fibers (36). Therefore, the lack of significant changes in these genes expressions in the current study might be because of specific characteristics of the myocardium tissue.

VEGF expression is primarily regulated by the stabilization of HIF-1 via HIF-1α translocation in response to hypoxic conditions (18). In our study, HIF-1α remained consistently unchanged alongside VEGF across all groups. Reduced intracellular oxygen tension has been suggested as a key factor inducing HIF-1α and its downstream target VEGF mRNA expression (35, 36). Consequently, upregulation of HIF-1α and VEGF mRNA and protein expression has been observed in acute ischemic conditions compared to nondetectable levels in the normal ventricles (37). It is unlikely that myocardium develops hypoxic conditions during exercise since the increased oxygen demands during exercise is sufficiently met by 5 fold coronary blood flow (38). Therefore, the unchanged levels of HIF-1α and VEGF in our study might be attributed to the maintained intracellular oxygen tension through increased coronary blood flow. These results may suggest that the increased oxygen demands during exercise is efficiently met by oxygen supply in the diabetic and nondiabetic rats, highlighting the responsiveness of the myocardium to exercise-induced stress, irrespective of the diabetic status.

Exercise training has been consistently shown to enhance the myocardial vascular growth and arteriolarization of the coronary capillaries (39). In addition, in the diabetic heart, exercise training was also shown to improve coronary blood flow and reverse the increased vascular resistance (40). These adaptations in cardiac vasculature function has been, at least partially, attributed to be mediated through VEGF and other growth factors (41). However, our findings showed no increase in the HIF-1α and VEGF expression, suggesting limited adaptations to the cardiac vasculature through this mechanism, had the exercise been performed for longer term. alternatively, vascular angiogenesis may occur through different mechanisms other than increased VEGF, such as enhanced expression of the VEGF receptors Flt1 and Flk1 (42). Unfortunately, the acute responses of those receptors to exercise have not been studied in normal and diabetic hearts.

Exercise surprisingly induced immediate upregulation in TSP-1, an anti-angiogenic factor (43). This acute response in the myocardium has not been examined. However, some studies assessed the acute response of TSP-1 mRNA expression to a single exercise session in skeletal muscles (44, 45). Following one hour of treadmill running, skeletal muscles of mice exhibited a ~ six-fold increase in TSP-1 mRNA expression 1 h post-exercise, similar to our findings in the myocardium. However, with short term of exercise training (3 – 5 days), the acute response of TSP-1 mRNA to exercise was abolished, and returned to increase ~ 3.4-fold after 8 weeks of training (44). In another study, 1 h of treadmill running increased TSP-1 mRNA expression by 2.5 – 3-fold 3 hours post-exercise in the skeletal muscles of STZ-induced diabetic mice (46). These findings in the skeletal muscles align with the acute myocardial TSP-1 response to a single running exercise session observed in our study. However, the long-term effects of exercise training on the myocardial response remain unknown. It was expected that exercise would exert an inhibitory effect on TSP-1 to facilitate angiogenesis and enhance tissue capillarization. Unfortunately, there are limited data available for comparison with our findings in regard to the myocardial response of TSP-1 to exercise. It was suggested that TSP-1 is associated with decreased cardiac inflammation and scaring during ischemia/reperfusion injury in the cardiac muscles (47). This implies a potential role of TSP-1 in mitigating the inflammatory response during stressful cardiac events, such as exercise, other than its anti-

angiogenic role. Nonetheless, the TSP-1 acute response to exercise in the myocardium remains largely unknown.

We observed a greater PGC-1α mRNA in the DIS, which was further increased with exercise in the DIEX and CEX. The effects of diabetes on the myocardial PGC-1α have been shown with mixed findings. Mitochondrial content, DNA content, and proteins of the respiratory chain of oxidative phosphorylation were increased, but with reduced mitochondrial respiration and function in the diabetic heart (12, 13). In agreement with our findings, in these stages of diabetes, mitochondrial biogenesis was associated with increased expression of PGC-1α (13, 48). However, PGC-1α upregulation might have been transient, possibly due to increased fatty acids availability within the diabetic myocardium (13, 49). This temporal phenomenon was demonstrated by findings showing an initial increase in PGC-1α mRNA at the earlier stages of diabetes followed by a subsequent decrease at later stages in the diabetic hearts (14, 50).

Chronic and acute exercise have been shown to consistently increase PGC-1α mRNA and protein expression in the diabetic and non-diabetic skeletal muscles of human and animal models (23, 51, 52). However, data on acute cardiac response is scarce, both in the presence or absence of diabetes. Myocardial PGC-1α expression in response to exercise has been mainly studied during longer terms of aerobic exercise (3 – 10 weeks) in diabetic and non-diabetic rats (9). In normal rats, 10 weeks of treadmill running training increased the myocardial PGC-1α protein but not mRNA expression (9). On the other hand, diabetic hearts increased both PGC-1α mRNA and protein content in the myocardium compared to no increase in the normal hearts of aged rats following 3 weeks of running training (53). In the present study, both diabetic and non-diabetic hearts exhibited a similar increase in PGC-1α mRNA expression. Notably, our animals were ~ 12 weeks old and STZ-induced diabetic, compared to 8 months old and obesity-induced diabetic, respectively, in the study of Botta *et al.* (2013) (53). Nonetheless, the current findings suggest that the diabetic heart may develop comparable rate of cardiac nuclear-encoded mitochondrial genes adaptations to exercise training as observed in normal hearts. Nevertheless, the question remains whether these similar responses of PGC-1α mRNA to acute exercise between diabetic and non-diabetic hearts translate to comparable increases at the protein level and subsequent enhanced mitochondrial content and function. Further studies are highly warranted to elucidate the longer-term effects, with a primary focus on mitochondrial respiration, content, and overall diabetic heart function exposed to exercise training.

Since mitochondrial dysfunction in diabetic cardiomyopathy has been linked to increased oxidative stress in the diabetic heart, we speculated that exercise would result in increased MDA in DIEX relative to CEX. However, they both induced similar levels of lipid peroxidation. Diabetic cardiomyopathy has shown an increased levels of MDA production compared to non-diabetic heart (54), and exercise training reduced the systemic and cardiac MDA in diabetic myocardium (55-57). The utilized exercise intensity in our protocol may explain the absence of MDA changes. Nonetheless, this type of exercise has been shown to improve cardioprotection and functionality (58). Given MDA was not further increased in DIEX, this suggests that diabetic cardiomyopathy may benefit from this type of exercise without exacerbating the levels of oxidative stress.

4.1. Limitations and future directions

We have included the mRNA expression data only. Unfortunately, we could not include the western blot data due to excessive delay in obtaining the required materials. Given the diabetic and non-diabetic myocardium demonstrated similar mRNA transcriptions, assessing the level of protein expression would have provided essential insights into whether these transcripts translate to corresponding protein expression. It is unclear with the limited current data to determine if both diabetic and non-diabetic hearts would exhibit similar or different post-transcriptional regulation or protein synthesis. In addition, baseline assessment of myocellular protein abundance could be better incorporated in the interpretation of our results. For example, it is possible that if the non-diabetic myocardium had higher baseline PGC-1α protein abundance, it would have a diminished mRNA expression response to exercise, rather than comparable response to the diabetic heart. In addition, we lacked the right equipment to verify diastolic dysfunction or other functional signs of diabetic cardiomyopathy. However, similar rat strain was shown to develop diabetic cardiomyopathy within 4 – 6 weeks from diabetes induction. To ensure cardiomyopathy, we mitigated this limitation by following similar procedures, including the STZ dosage, the rat strain, and the duration following diabetes induction (25, 26).

Future studies should incorporate measuring protein expression of the critical proteins, such as PGC-1α and VEGF, at the baseline and in response to exercise. In

addition, the responses of other mitochondrial function markers to exercise should be assessed, including mitochondrial fusion and fissions proteins, key regulators of mitochondrial efficiency such as uncoupling proteins, and measures of mitochondrial oxidative stress. Furthermore, the therapeutic implications of the current findings should be considered provisional until further studies explore the long-term effects of this type of exercise on the diabetic myocardium vascularization, mitochondrial function, and myocyte respiration. Moreover, cardiac function, including diastolic function, and whether these improvements are comparable between the diabetic and non-diabetic hearts, as suggested by the current acute molecular responses. In addition, future studies should aim to evaluate these exercise interventions and their responses at the acute and long-term phases across various diabetic phenotypes in humans.

5. Conclusions

Our findings suggest that a single bout of moderate exercise induces similar mitochondrial biogenic and vascular angiogenic molecular responses in the diabetic and non-diabetic myocardium. These findings imply that the diabetic heart may develop comparable rates of cardiac adaptations in response to exercise training as the normal heart, providing preliminary evidence for potential therapeutic implications for managing the diabetic heart. Nonetheless, more sophisticated investigations are warranted to examine the longer-term of this type of exercise on the mitochondrial content and function, vascularization, and overall cardiac function improvements. It remains unknown whether these acute responses would be functionally translated to same levels in the diabetic and nondiabetic hearts.

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Availability of Data and Materials

Data are available upon request from the corresponding author.

Ethical Approval and Consent to Participate

All procedures were approved by local committee at Yarmouk University, Jordan following the Guide for the Care and Use of Laboratory Animals (IACUC/2020/2).

Consent for Publication

None.

Competing Interests

The authors have no conflict of interests or any disclosure to report.

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Authors' Contributions

Conceptualization, RAA.; methodology, RAA, BAS, SH, TAM, and BAT; software, RAA, TAM, ROA, EAA, MAM, and ASB; validation, RAA, BAT and BAS; formal analysis, RAA, BAS, ROA; investigation, RAA, BAS, SH, TAM, ROA, EAA, MAM, and ASB; resources, RAA and BAT; data curation, RAA, BAS, SH, TAM, ROA, EAA, and MAM; writing—original draft preparation, RAA; writing—review and editing, RAA, BAS, BAT; visualization, RAA, SH; supervision, RAA, BAT; project administration, RAA, BAT; funding acquisition, RAA and BAT. All authors have read and agreed to the published version of the manuscript.

Declaration

None.

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