The effect of resistance exercise on oxidative stress in cardiac and skeletal muscle tissues of streptozotocin-induced diabetic rats

Ali Samadi [1*], Abbasali Gaeini [2], Aliasghar Ravasi [2], Mehdi Hedayati [3], Mostafa Rahimi [4]

1. Department of Physical Education and Sport Science, Faculty of Humanities, Shahed University, Tehran, Iran.
2. Department of Exercise Physiology, Faculty of Physical Education and Sport Science, University of Tehran, Tehran, Iran.
3. Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
4. Ph.D Student in Exercise Physiology, Exercise Physiology Research Center, Baqiyatallah University of Medical Science, Tehran, Iran.

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A B S T R A C T

Background and Objective: It has been shown that oxidative stress increases in diabetes and it has an important role in its development and subsequent complications. Thus, the aim of this study was to investigate the effect of acute resistance exercise on oxidative stress in skeletal muscle and cardiac tissues of streptozotocin-induced diabetic rats.

Materials and Methods: Twenty male wistar rats were rendered diabetic by a single dose of streptozotocin (STZ; 50 mg/kg, IP) and were randomly divided into two groups: (1) acute resistance exercise and (2) sedentary control. Acute resistance exercise consisted of 4 separate sessions of exercise that happened in non-consecutive days. After the last session, the animals were anesthetized by xylazine (10 mg/kg) and ketamine (75 mg/kg) and flexor hallucis longus (FHL) muscle and heart were surgically removed and stored at -80 °C until biochemical analysis of malondialdehyde (MDA), protein carbonyl (PC), and glutathione (GSH) was done.

Results: Our findings showed a significant decrease of MDA (p=0.007), but not PC level (p=0.678) of cardiac tissue of resistance exercise group. However, in FHL muscle, resistance exercise caused a significant increase in MDA (p=0.01), but there was no significant changes in PC level (p=0.399). Resistance exercise also caused a small but insignificant increase in GSH content of both skeletal and cardiac muscle tissues (p=0.11 and p=0.19, respectively).

Conclusion: We observed that in diabetic rats, acute resistance exercise decreases cardiac tissue MDA, increases skeletal muscle MDA level, and had no significant effect on PC and GSH level. Further research is needed to specify the mechanisms of these differences in various tissues following resistance exercise.

Key Words:
Oxidative Stress
Resistance exercise
Streptozotocin
Diabetes mellitus

1. Introduction

Diabetes mellitus (DM) is a significant health concern all over the world. It affects 285 million people leading to cardiovascular disease, nephropathy, retinopathy, and widespread disease of both the peripheral and central nervous systems (1, 2). Also, it is predicted that by the year 2030, 439 million individuals will be afflicted with DM and its debilitating consequences (2). About the pathology of diabetes, it has been believed that insulin resistance in muscle and liver and beta cell failure are the core pathophysiologic defects (3).

Hyperglycemia and hyperlipidemia are key promoters of diabetes dysmetabolism, namely,

[*]**Corresponding Author:**
Ali Samadi
Department of Physical Education and Sport Science, Faculty of Humanities, Shahed University.
E-mail: ali.samadi.62@gmail.com Tel: 09127996853

through the formation of advanced glycation end products (AGEs) and reactive oxygen species (ROS) which lead to oxidative stress, and consequently causes cell damage and insulin resistance (4, 5). Oxidative stress is a condition in which the equilibrium between free radicals production and their scavenging by the antioxidants becomes perturbed and leads to accumulation of free radicals or their products within cells (6).

Oxidative stress has been shown to be involved in triggering cardiomyocyte apoptosis associated with diabetic cardiomyopathy. Moreover, some recent investigations have been proposed that DM causes an inflammatory response through the oxidative mechanisms, which may play a causative role in development of diabetic cardiomyopathy (7). At skeletal muscle level, it has been documented that oxidative stress results in oxidative damage of muscle proteins that in turn may result in protein denaturation, aggregation, and loss of essential biological function (8). As studies have shown, skeletal muscle accounts for the majority of insulin-stimulated glucose uptake and that >80% of glucose is then stored as glycogen. The rate of glycogen synthesis in skeletal muscle was ≈50% lower in diabetic subjects than in normal volunteers (9). In fact, oxidative stress is believed to modify a number of the signaling pathways involved in insulin signaling. It forms the foundation for the induction of multiple cellular pathways, primarily mitogen-activated protein kinases (MAPK) pathways, which can ultimately lead to both the onset and subsequent complications of DM (1, 10, 11).

For decades, the three cornerstones of medical care in diabetes have been diet, medication, and exercise. In fact, regular exercise has been shown to improve blood glucose control, reduce cardiovascular risk factors, contribute to weight loss, and improve well-being (12). Moreover, considering the reports about the positive effect of resistance exercise on insulin sensitivity, glycemic control, body composition, muscle mass and quality, and cardiac function nowadays both American diabetes association and American heart association have put resistance type exercise in exercise recommendations for diabetics (9, 12).

Regarding to the role of oxidative stress in diabetes complications and cell signaling pathways, plenty of studies have investigated the effect of different modalities of exercises, mostly aerobic exercises, on oxidative stress in diabetic organs and has reported different results (4, 11, 13-15). Lack of consistency in findings may be due to differences in type, intensity and duration of exercise protocol used in these studies. However, less is known about the effect of acute resistance exercise on the oxidative stress in diabetic organs, especially cardiac and skeletal muscle tissues. Since exercise is often prescribed as a non-pharmacologic treatment of diabetes, the aim of the present study was to investigate the effect of acute resistance exercise on oxidative stress in cardiac and skeletal muscle tissues of STZ-induced diabetic rats.

2. Materials and Methods

All experiments involving the animals were conducted according to the policies of the Iranian Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes. Twenty male Wistar rats, 6-8 weeks of age and weighing 140±10 g, were obtained from Pasture Institute of Iran. They were housed in temperature and humidity controlled environment at 22±3 °C with a 12:12 light-dark cycle with the lights on at 7 a.m. and off at 19 p.m. Rats were fed a standard rodent diet (16). After 3 weeks of adaptation to new environment, diabetes was induced and animals were randomly divided into two groups: (1) acute resistance exercise (RE, n=10), (2) sedentary control (C, n=10) (Table 1). The sedentary group rats remained in their cages for the entire duration of the experiment. Two animals from each group fall out of the study because of severe weight loss and death.

2.1. Induction of diabetes

Diabetes was induced with a single intraperitoneal injection of freshly prepared streptozotocin (50 mg/kg; Alexis) in 1 ml of citrate buffer (pH 4.5) (16). Blood glucose levels were determined 4 days after STZ injection using a blood glucose tester (Gluco plus, Canada). Animals with blood glucose level ≥300 mg/dl were used as the diabetic (Table 1). Changes in body weights and blood glucose before and after exercise protocol have been summarized in Table 1.

Table 1. Mean weight (g) and blood glucose concentrations (mg/dl) in experimental groups.

Group	Weight pre	Weight post	Glucose pre	Glucose post
RE	213±10	198±16	321±17	245±31
C	216±13	213±14	331±28	227±33

* The values represent mean±SD of 8 animals per group. RE: Resistance, Exercise, C: Control.

2.2. Resistance exercise

Acute resistance exercise consisted of four separate exercise sessions in non-consecutive days. An exercise consisting of climbing a 1 m ladder with a 2 cm grid ladder and weights attached to the rats' tails was used as resistance exercise (17). On day 1, to introduce the rats with exercise procedure they performed 10 climbing without carrying any weight. On day 2, they carried light weights 0.2 to 0.5 of their body weight ~10 climbing. On day 3, maximum carrying capacity of rats was measured according to following procedure. Animals performed 4-6 repetitions with carrying weights 0.2 to 0.5 of their body weight (18). Then, weights gradually (30 g) increased until the rats reached to exhaustion. The criterion for the exhaustion was the animal's refusal from climbing after three intense stimulation of the tail. No electrical shock was used in this study (19). On day 4, rats preformed 10 climbing with 70-75 percent of their maximum carrying capacity with a 1.5 min rest interval among the reps, when the rats reached the top of the ladder; they were allowed to recover in the resting area (17).

2.3. Tissue and blood sampling

All samples were taken after overnight fasting. Rats were anesthetized with an intraperitoneal injection of xylazine (10 mg/kg) and ketamine (75 mg/kg) after exercise (i.e., 4-5 min) before sampling (19). Surgical removal of heart and flexor hallucis longus (FHL) muscle for analysis was finished within 15 min, then they were washed in saline and stored at -80 °C; blood was completely collected via cardiac puncture after the rats were anesthetized. Blood was transferred to a microtube where it was allowed to clot (15-20 min) before being centrifuged (3500 rpm for 15 min), and the serum was collected and stored at -80 °C until were used for biochemical analysis.

2.4. Sample preparation

Tissues including FHL muscle and heart were homogenized by homogenizer (Miccra, Germany) in antiprotease containing buffer (PBS, pH=7.4, 0.1 M, contained problock cocktail as antiprotease cocktail, GOLDBIO, USA) for ~ 45 s at second speed level (10.000 min-1). Then, the homogenates were centrifuged at 3000 rpm for 10 min at 4 °C (Hettich Mikro 200R, Austria) and the supernatant was used for analysis.

2.5. Biochemical analysis

Malondialdehyde (MDA), an important indicator of lipid peroxidation, was determined photometrically by thiobarbituric acid-reactive-substances (TBARS) assay method in homogenates according to kit instructions (Photometric, BioAssay Systems, CA, USA). Protein carbonyls (PC), an indicator of protein oxidation, were assessed calorimetrically by measuring carbonyl formation using 2, 4-dinitrophenylhydrazine (DNPH) as a reagent according to kit instructions (Cayman, MI, USA). Skeletal and cardiac muscle level of total glutathione content (GSH) was evaluated by the GR recycling method adapted for the microplate reader using the colorimetric method as directed by the kit instructions (Cayman, MI, USA). Moreover, blood glucose was assayed using colorimetric enzymatic method (Parsazmun, Tehran, Iran) on a Tecan microplate reader at 546 nm.

2.6. Statistical analysis

All data were expressed as mean±SD. A Student t test was used to determine differences between exercise and control groups. The level of significance was set at $p \leq 0.05$.

3. Results

3.1. MDA and PC Level

Skeletal muscle MDA significantly increased in exercise group as compared to control group (p=0.01) and it significantly decreased in the cardiac muscle in comparison with control group (p=0.007). There was also no difference between exercise and control group regarding PC level in skeletal and cardiac muscle tissues (p=0.399 and p=0.678, respectively) (Table 2).

3.2. GSH Content

In exercise group, there was a slight increase in skeletal and cardiac muscle GSH content but it was not significant (p=0.11 and p=0.19, respectively).

Table 2. The effect of exercise on skeletal and cardiac muscle MDA and PC levels.

Group	Tissue	MDA (nmol/g tissue)	PC (nmol/g tissue)
RE	FHL Muscle	8.72±1.15 [a]	2.53±0.7
C		4.71±3.42	2.79±0.45
RE	Heart	4.30±0.70 [b]	1.81±0.58
C		5.73±1.08	1.97±0.89

* The values represent mean±SD of 8 animals per group. RE: Resistance Exercise.C: Control. a: significant increase. b: significant decrease.

Table 3. The effect of exercise on skeletal and cardiac muscle GSH content.

Group	Tissue	GSH (nmol/g tissue)
RE	FHL Muscle	1.20±0.20
C		1.07±0.08
RE	Heart	1.77±0.15
C		1.65±0.19

* The values represent mean±SD of 8 animals per group. RE: Resistance Exercise.C: Control.

4. Discussion

In this study we investigated the effect of acute resistance exercise on cardiac and skeletal muscle tissues oxidative stress in STZ-induced diabetic rats. The main findings of our study were: i) acute resistance exercise decreases cardiac oxidative stress, as was obvious by lower level of MDA, but it also lead to an increase in skeletal muscle oxidative stress as was obvious by a significant increase in MDA level; ii) acute resistance exercise also causes a slight but non-significant increase of skeletal and cardiac muscle GSH content.

Medical and scientific community are interested in the effects of exercise in diabetic organisms. Although some studies have surveyed the effect of acute and chronic endurance exercise on oxidative stress in different diabetic organs (4, 11, 13-15), to our knowledge, this is the first study investigating the effect of acute resistance exercise on cardiac and skeletal muscle oxidative stress of diabetic rats. Numerous studies have reported increases in various oxidative stress biomarkers in several tissues following aerobic exercise protocols (4). Our results showed that acute resistance exercise significantly augments the already elevated muscle MDA levels in skeletal muscle of exercise group versus the sedentary control group. These results suggest that the intensity and duration of the resistance exercise was able to exceed the antioxidant defences of skeletal muscle.

Consistent with our findings, Bejma and Ji found out that an acute bout of exercise resulted in a significant increase in muscle lipid peroxidation, but there was not any significant alteration in PC level (21). Ghosh et al reported increased levels of PC and lipid hydroperoxides, products of protein and lipid peroxidation, after exercise in diabetic mice (20). Goldfarb et al showed that moderate resistance exercise (70% 1RM) in arm resulted in a significant increase in PC level immediately and 15 min after exercise but light resistance exercise (30% 1RM) did not elicit a significant increase in PC in healthy subjects (22). Although the exact mechanism of increased ROS during resistance exercise is not fully elucidated, mechanism for the increase in ROS production with resistance exercise could be similar to that seen in ischemia-reperfusion injury. Moderate to intense contraction is associated with alternative periods of ischemia-reperfusion in muscle. The ischaemic conditions trigger conversion of xanthine dehydrogenase to xanthine oxidase. Xanthine oxidase produces superoxide radical that is the most abundant free radical in the cell and leads to damage to cells macromolecules and subsequent increase in markers of protein and lipid proxidation (23). But, the significant increase in MDA level beside the insignificant changes in PC level suggesting the differences in susceptibility to free radicals invasion between protein and lipids.

However, our findings showed that resistance exercise actually decreases cardiac MDA and did not alter PC level in cardiac muscle tissue. Gul et al were found unaltered TBARS levels in male diabetic rats after exhaustive exercise (24). In contrast, Turgut et al reported an increased MDA level in heart tissue after swimming to exhaustion and also after 30 min of swimming in rats (25). Unchanged PC and decrease in lipid peroxidation marker (MDA) after resistance exercise found in our study suggests that resistance exercise may decrease oxidative stress in cardiac muscle or heart tissue should have a strong enzymatic and non-enzymatic antioxidant defence against exercise-induced oxidative process.

Our result also showed that resistance exercise did not improve antioxidant status in the skeletal and cardiac muscle, as measured by GSH content. But it did lead to slight increase in both skeletal and cardiac muscle GSH content. Both acute and chronic exercise has been shown to increase GSH level in rat skeletal muscle (26). On the other hand, exhaustive exercise has been reported to decrease total GSH content in the skeletal and cardiac muscle of rats (27). GSH is a major intracellular antioxidant molecule and constitutes an important mechanism against oxidative stress (11). It has been shown that acute exposure of tissue to hypertensive oxygen increases GSH content by several folds within 24 h (26). As contractile activity cause an increase in free radicals production (21), it is conceivable that repeated exposure of the cells to oxidative challenge will upregulate the antioxidant defense system to protect the cells against oxidative damage. However, in our study the amount of increase was not significant which may be because of exercise type and intensity or may be that the oxidative challenge made by exercise was not powerful enough to stimulate the cellular pathways such as NFκB and JNK, involved in upregulation of genes responsible for coding the enzymes which are essential for GSH biosynthesis.

In conclusion, the results of the present study revealed that resistance exercise decreases lipid proxidation in cardiac muscle tissue, as measured by MDA level, but it increased lipid proxidation in skeletal muscle and had no significant effect on PC level. Moreover, resistance exercise led to small but insignificant increase in skeletal and cardiac muscle GSH content. These results suggest that acute resistance exercise may have different effects on various tissues and further research is needed to determine the reasons of these differences and specify the underlying mechanisms.

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