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Effects of Acute Exhaustive Exercise on Oxidant and Antioxidant System Parameters in Rats with Streptozotocin Induced Diabetes Mellitus

Akut Tüketici Egzersizin Streptozotosinle Oluşturulmuş Diyabetik Rat Modelinde Oksidan ve Antioksidan Sistem Parametreleri Üzerine Etkisi

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ABSTRACT

Objective: The aim of this study was to investigate the changes in certain oxidative stress and antioxidant system parameters in response to acute exhaustive exercise (AEE) applied to diabetes-induced rats.

Methods: 16 male Wistar rats were divided diabetes (n=8) and control (n=8) groups. For diabetes induction, 65 mg/kg of streptozotocin (STZ) administered to rats intraperitoneally. On the fourth day of STZ administiration, blood glucose level was measured and level of above 180 mg/dL was considered as diabetes mellitus (DM). AEE procedure was applied to rats within the same day that they were considered as diabetes. After the exercise protocol, blood samples were collected for biochemical analyses and serum samples were obtained by centrifugation.

3-nitrotyrosine, LPx, protein carbonyl, SOD and GSH, GSH-Px levels were analyzed by using Enzyme-linked immunosorbent assay.

Results: After the exercise, both groups had decreased blood glucose levels compared to pre-exercise levels. There was a significant increase in lactate levels of both groups, compared to pre-exercise levels. In terms of distance run, control group ran approximately two times more distance than the diabetes group. When we compared oxidative stress parameters; Lipid peroxide, 3-nitrotyrosine, and protein carbonyl levels were significantly higher in the diabetes group. Antioxidant parameters; glutathione and glutathione peroxidase were significantly higher in the diabetes group. As for superoxide dismutase levels, it was slightly decreased in the diabetes group, but did not reach statistical significance.

Conclusions: AEE triggers both oxidant and antioxidant systems in diabetic rats.
Keywords: Diabetes Mellitus, acute exhaustive exercise, oxidative stress, antioxidants

ÖZ

Amaç: Çalışmamızın amacı; streptozotocinle oluşturulmuş diyabetik rat modelinde akut tüketici egzersizin oksidatif stres ve antioksidan sistem üzerine etkisini araştırmaktı.

Gereç ve Yöntemler: 16 erkek Wistar ratı diyabet oluşturulacak grup (n=8) ve kontrol grubu (n=8) olarak ikiye ayrıldı. Diyabet oluşturmak için 65 mg/kg streptozotosin (STZ) intraperitoneal olarak uygulandı. STZ uygulamasının dördüncü gününde kan glikoz seviyesi ölçüldü ve 180 mg/dl'nin üzerindeki değerler diyabetes mellitus (DM) olarak

kabul edildi. Diyabet oluşturulan bu ratlara aynı gün akut tüketici egzersiz yaptırıldı. Egzersiz bitiminde biyokimyasal analizler için kan alındıktan sonra santrifüj yapılarak serum örnekleri elde edildi. 3-nitrotyrosine, LPx, protein carbonyl, SOD ve GSH, GSH-Px seviyeleri "enzyme-linked immunosorbent assay" sistemi ile ölçüldü.

Bulgular: Her iki grupta egzersiz sonrası kan şekeri düzeyleri, egzersiz öncesi ile kıyaslandığında azalma gösterdi. Her iki grubun laktat seviyelerinde de egzersiz sonrasında artış tespit edildi. Koşu mesafesi bağlamında değerlendirildiğinde ise kontrol grubu diyabet grubundan yaklaşık iki kat fazla koştu. Oksidatif stress parametrelerinden lipit peroksit, 3-nitrotirozin ve protein karbonil seviyeleri diyabetik grupta anlamlı derecede artmıştı. Antioksidan parametreler olan glutatyon ve glutatyon peroksidaz da diyabetik grupta yine anlamlı olarak artış göstermişti. Süperoksit dismutaz seviyesinde diyabetik grupta hafif bir azalma olduysa da istatistiksel olarak anlamlılık tespit edilmedi.

Sonuç: Akut tüketici egzersiz diyabetik ratlarda hem oksidan hem de antioksidan sistemi harekete geçirmektedir.

Anahtar Sözcükler: Diabetes Mellitus, akut tüketici egzersiz, oksidatif stres, antioksidanlar

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INTRODUCTION

Diabetes mellitus (DM) is one of the most common chronic diseases and its incidence increased rapidly in recent years. Both form of this disease (Type 1 and 2) cause and increase in oxidative stress (1,2). One of the effective method which is used both in reducing the risk of DM and for its treatment is regular exercise. However, it is obvious that the duration and intensity of the physical activites prescribed for diabetic patiens increase oxidative stress, as well. This is due to the increase in oxygen consumption during exercise which also leads to leaks in the mitochondrial respiratory chain that result in an increase of certain reactive oxygen species (ROS) such as superoxide and hydroxyl radicals (3, 4). On the other hand, regular and long-term exercises have been shown to reduce free radical damage and enhance the antioxidant defense (5-8). It has been shown that thiobarbituric acid reactive subtance (TBARS) level in the vastus lateralis is decreased and Glutathion Peroxidase (GSH-Px) level in gastrocneimus muscle is increased with endurance type training in streptozotocin (STZ) induced DM rats (9).

The intensity and the duration of the exercise has a great importance in means of oxidant-antioxidant balance of DM patients. Although regular exercise has been shown to reduce oxidative stress in DM; the effect of acute exhaustive exercise (AEE) on oxidative stress is not well described. Blood lactate levels increase during exercise depending on the intensity, as well (10, 11, 13 ,14). In this context, this study was planned to understand the metabolic responses to overloading. We also believe that our findings may shed light on a popular training method (high intensity interval training method) in which exercise intensity could be increased to maximum levels as in acute exhaustive exercise (10). In the present study, we aimed to investigate the effects of AEE on oxidative stress parameters [lipid peroxide (LPx), protein carbonyl, 3-nitrotyrosine], antioxidant system markers [superoxide dismutase (SOD), glutathione (GSH), GSH-Px] and the lactate levels in STZ induced diabetic rats.

METHODS

All protocols of the study was reviewed and approved by the Istanbul University Ethical Committee (report no: 2013/78, date: 17/08/2013). 16 male Wistar albino rats (weight: 289-344 g) were used. The rats were housed in standard cage and ambient conditions with 12 hours light, 12 hours dark principle. Food and water were not restricted. After room adaptation, two groups were formed as experimental group (n = 8) and control group (n = 8). Randomisation was not implemented as it was predicted that the experimental group could experience more weight loss after the diabetes induction.

To induce DM in experimental group rats, a single dose of 65 mg/kg STZ (Sigma) was administered intraperitoneally in 1 mL of saline (11-13). The rats were weighed before STZ administration and following exercise (Table 1).

Table 1. Body weights of rats in the experimental group and control group

Body Weight (grams)	Experimental Group (mean ± SD)	Control Group (mean ± SD)
Before STZ	344.63±12.81*	289.50±3.58
After STZ	276.25±18.02†	290.75±3.25

**=Experimental group vs control group before STZ (p<0,005), †= Experimental group before STZ vs After STZ (p<0,05). STZ: Streptozotocin.*

Prior to STZ administration, blood glucose and lactate levels of both groups were measured. Abbott FreeStyle Optium (USA,2015) was used for blood glucose measurement and EDGE ApexBio (Taiwan,2015) was used for lactate measurement. Blood samples were collected from the tail. On the fourth (diabetes group) and fifth day (control group) of STZ administiration, blood glucose and lactate levels were measured and glucose levels of above 180 mg/dL were considered as DM in experimental group. AEE procedure was applied to both groups within the same day. Following AEE, blood glucose and lactate levels were measured (Tables 2).

Table 2. Blood glucose and lactate levels of the experimental and control group rats

	Experimental Group (mean ± SD)			Control Group (mean ± SD)		
	Before STZ	**After STZ**		**Before STZ**	**After STZ**	
		Before AEE	**After AEE**		**Before AEE**	**After AEE**
Glucose (mg/dL)	106.75±3.36	416.00±26.32 *,†	389.75±47.11‡,§	100.25±3.47	101.13±2.40	76.63±13.89
Lactate (mg/dL)	23.50±2.16	46,00±9.49	161,13±2.68¥,¤,#	25,38±4.83	24,63±4.16	73,13±14.16ϑ,ʒ

**: Experimental group before AEE vs control group before AEE (p<0.005), †: Experimental group before AEE vs before STZ (p<0.001), ‡: Experimental group after AEE vs control group after AEE (p<0.005), §: Experimental group after AEE vs before STZ (p<0.005). STZ: Streptozotocin; AEE: Acute Exhaustive Exercise. ¥: Experimental group after AEE vs before AEE (p<0.001), ¤: Experimental group after AEE vs before STZ (p<0.001), #: Experimental group after AEE vs control group after AEE (p<0.005), ϑ: control group after AEE vs before STZ (p<0.05), ʒ: Control group after AEE vs before AEE (p<0.05). STZ: Streptozotocin; AEE: Acute Exhaustive Exercise*

Both groups ran on a treadmill 10 min/day for consecutive 2 days before experimental protocol was applied, at a speed of 10 m/min for familiarization. AEE protocol was implemented on a motorized treadmill at 20 m/min of speed until exhaustion. For running induction, electrical stimulation (20-40 volts) was delivered to rats via a stimulator (14). Despite all physical and electrical stimuli, absence of any activity or immobilization on the treadmill was considered as exhaustion and the running process was terminated. After the exercise protocol, blood samples were collected in vacutainer tubes intracardially under ether anesthesia and immediately transported to the laboratory on ice. Serum samples were obtained by centrifugation (+4 °C, 3000 rpm, 10 min). Samples were stored at –80 °C until assayed for levels of the markers. Finally, the rats were sacrificed by cervical dislocation.

3-nitrotyrosine, LPO, protein carbonyl, SOD and GSH, GSH-Px levels were analyzed by using Enzyme-linked immunosorbent assay (EIAab Sci-

ence, Wuhan, China). GSH (EIAab, Catalog no:E0596r), GSH-Px (EIAab, Catalog no:E0295r), 3-nitrotyrosine (EIAab, Catalog no:E1863r) and SOD (EIAab, Catalog no:E0596r) ELISA methods are based on the competitive binding enzyme immunoassay technique. Results of GSH and 3-nitrotyrosine are expressed in ng/mL. Units of 3-nitrotyrosine GSH-Px and SOD are expressed in ng/mL. Protein carbonyl assay kit (Cayman, Catalog no:10005020) utilizes the DNPH reaction to measure the protein content in plasma, serum, cell lysates or tissue homogenates in a convenient 96-well format. This assay works best when samples have protein concentrations in the range of 1-10 mg/mL. LPx ELISA kit (My-BioSource, Catalog no: MBS763367) was based on sandwich enzyme-linked immune-sorbent assay technology. The limits of analytes are taken as follows: GSH : 1.25-80.0 ng/mL, protein carbonyl: 1-10 mg/mL, GSH-Px: 31.2 U/mL - 2000 U/mL, 3-nitrotyrosine: 1.56-100 ng/mL, SOD: 1.56-100 U/mL, LPx: 3.125-200ng/ml.

The SPSS version 13.0 program was used for the statistical analyses. For intra-group comparison Wilcoxon Rank Test, for intergroup comparison Mann-Whitney U Test were used. Repeated measurements of lactate and glucose changes were analyzed by Bonferroni Test, following ANOVA variance analysis. In all tests, $p < 0.05$ was considered as statistically significant.

RESULTS

At the beginning of the study experimental group's body weight was significantly higher than the control group before STZ administration. The body weight of the experimental group decreased significantly following STZ administration (Table 1). There was no significant difference between the weight of the control group rats and experimental group rats before the exercise.

There was no significant difference in glucose levels between groups before STZ administration. STZ administration led a significant increase in the glucose level when compared to measurements performed before and after AEE in experimental group (Table 2). Glucose level of the experimental group before AEE and after AEE was also found to be significantly increased when compared to control group before and after AEE (Table 2).

Lactate level of the experimental group was higher than control group after AEE (Table 2). In experimental group, lactate level after AEE was significantly higher when compared to levels before STZ and AEE (Table 2).

In the control group, lactate level after AEE was higher than before STZ and AEE levels (Table 2). Following AEE, the running distance of experimental group was significantly lower than control group (Table 3).

Table 3. Comparison of oxidative stress, antioxidant parameters and running distance after AEE

Biochemical Parameters	Experimental Group (mean±SD)	Control Group (mean±SD)	P value
3-Nitrotyrosine (pmol/ml)	80.19±4.25	60.61±1.52.	p< 0,005
LPx (nmol/ml)	1.74±0.39	1.60±0.25	NS
Protein Carbonyl (pmol/ml)	1.76±0.25	1.41±0.19	p<0,05
GSH (pg/ml)	104.93±1.97	97.97±0.86	p<0,05
GSH-Px (pg/ml)	10.36±0.59	5.17±0.67	p<0,005
SOD (U/ml)	21.53±1.03	25.04±3.96	NS
Running distance (meter) at AEE	655.21±81.05	1351.46±142.42	p<0,01

NS= Not significant; LPx: Lipid Peroxide; GSH: Glutathione; GSH-Px: Glutathion Peroxidase; SOD: Superoxide Dismutase, AEE: Acute Exhaustive Exercise.

3-nitrotyrosine, protein carbonyl, GSH and GSH-Px levels of the experimental group were higher than the levels of control group. Although the LPx level of the experimental group was increased, it was not significant and the SOD level was somewhat decreased, without any statistical significance (Table 3).

DISCUSSION

High-intensity physical activities could be considered as a part of daily life. For instance, children can reach high-intensity levels of effort during their spontaneous playtimes, whereas adults can reach those levels usually in cases of emergency (15). Additionally, the intensity of exercise can rise up to similar levels as acute exhaustive exercise in high-intensity interval training programs which are increasingly used for patients with chronic diseases, such as diabetes mellitus (16). However, this type of exercises are recommended to diabetic patients who have well regulated blood glucose levels or to patients who have no complications of the disease.

It was shown that exhaustive exercise causes a 2-3 fold increase in ROS (17). In recent years, it was shown that exhaustive exercise caused an increase in lipid peroxidation, protein oxidation and DNA damage (18-21). On the other hand, regular exercise enhances the antioxidant status of the body (22,23). It was reported that on the third day of exhaustive exercise, malondialdehyde (MDA) levels were lower and GSH levels were higher in trained individuals when compared to sedentary people (24). According to the results of these studies, it is understood that it is more beneficial to perform an exercise that is adjusted according to the condition level of the individual and also it should be performed regularly. In other words, exercise induced oxidative stress may be balanced by enhancing the antioxidant systems through appropriate exercise programs.

Oxidative stress and in particular, lipid peroxidation increases significantly in DM (25,26). As well as DM itself, exercise programs applied to patients with DM increased plasma TBARS levels significantly (27). In this context, changes in oxidant-antioxidant status, particularly in response to acute exercise may provide insights into exercise prescription in DM, however the topic needs more investigation. Therefore, the aim of this study was to investigate the effects of AEE on the oxidative stress and antioxidant status in DM.

In our study, AEE led a significant increase in the oxidative stress parameters such as 3-nitrotyrosine, LPx and protein carbonyl in experimental group rats compared to the control group (Table 5). GSH and GSH-Px levels were found to be increased significantly in experimental group. On the contrary to our findings, decreased GPx levels were also reported in samples taken from sedentary and diabetic rat hearts after acute exhaustive exercise (28, 29). In addition, Somani et al. showed that acute exercise decreased GSH levels in rat liver and muscle but increased in the heart tissue. They also found that this increment in GSH levels was larger after acute exercise when compared to chronic exercise (30). On the other hand, there is also a study that reported no significant alteration in GPx levels after acute exercise in rat hearts. (31). These controversial results may be due to different methods used both in exercise model (low vs. high intensity, running vs, swimming, etc.) or evaluation of samples (heart, liver, muscle tissues, etc.) in these studies (Table 3).

In our study, lactate levels were significantly increased in diabetes group compared to non-diabetic controls. This increase in lactate levels may be due to altered intracellular glucose metabolism in diabetes. In recent years, increased lactate levels were found to be associated with serious health problems such as cancer or even in the diabetes prognosis (15). Although resistance type exercises are recommended for diabetic patients, negative effects of increased lactate levels encountered in these types of exercises should be taken into account.

SOD levels were found to be decreased in experimental group; however, this decrease was not significant (Table 3). Similar non-significant decrease in SOD levels reported in diabetes melli-

tus (32). Abou-Seif et al. showed a significant decrease in SOD levels in DM patients (33). Increased free radicals may oxidize and denature SOD enzyme. Also, the hyperglycemic process may lead to glycation and inhibition of SOD enzyme (34,35). In a study, an important antioxidant enzyme; paraoxonase-1 (PON1) activity was found to be increased significantly when compared to the control group in DM rats that were involved in a moderate and long-term exercise program. In the same study, conjugated dienes and TBARS levels which indicated oxidative stress and lipid peroxidation were significantly lower (36). Serum MDA levels were also found to be significantly decreased in DM rats that performed chronic exercise, however MDA levels were significantly increased in response to acute exercise in the same study (37). According to the current literature, if the exercise program is aerobic, moderate, long term, and regular, the oxidative stress parameters are lower than antioxidants and their balance changes in favor of antioxidant system. On the other hand, the number of studies investigating the effects of AEE on oxidant/antioxidant systems in DM patients are not sufficient.

CONCLUSIONS

In the present study, the changes in oxidant parameters such as nitrotyrosine, LPx and protein carbonyl in experimantal group are consistent with the literature findings. However, the increment of GSH and GSH-Px levels in experimental rats may be due to adaptive response to oxidative stress which is increased by acute exhaustive exercise.

In this context, we may suggest that AEE triggers both oxidant and antioxidant systems in DM rats. However, as for the experimental group, in case of blood glucose levels higher than 250 mg/dl, ketoacidosis risk arises. Therefore, in diabetic patients, blood glucose levels should be followed closely in such conditions.

Although studies on DM, oxidative stress and AEE provided many clues, the number of studies examining these three concepts together is limited and the results obtained in these studies vary. Even though we may evaluate some basic points, it is obvious that we do not have enough evidence to make a definite and clear comment. Forthcoming studies should consider the variable conditions such as the type of exercise and the timing of blood and/or tissue sampling.

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