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The Effect of Eccentric Exercise on Total Anti-Oxidant Capacity, Reduced Glutathione and Malondialdehyde Levels in Active Women

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Abstract

***Background*:** Although exercise can increase free radicals by generating oxidative stress, it also can decrease them by increasing the antioxidant enzymes in the body as well. The purpose of this study is to investigate the eccentric activity on some oxidative and anti-oxidative factors pertaining to blood plasma of PE women immediately after the exercise.

***Materials and Methods*:** Sixteen female students have been volunteered in this study randomly divided into two groups including eccentric training group and control group. The blood samples were drawn from the subjects one hour before and immediately after the exercise to measure the reduced Glutathione (GSH), Malondialdehyde (MDA) and total anti-oxidant capacity (TAC) levels. The data were analyzed by SPSS-13 software using the one-way analysis of variance, one-way ANOVA test, (to determine the differences between groups) at the confidence level of 90% ($p<0.05$).

***Results*:** The results has shown that the TAC, MDA, GSH levels after the eccentric exercise increased significantly compared to pre-exercise ($p=0.001$, $p=0.001$, $p=0.033$). The GSH and MDA levels also after the eccentric exercise were significantly higher than the pre-exercise compared to control group.

***Conclusion*:** It seems that sever eccentric exercise is an important stimulus making significant changes in body's anti-oxidative system and has the ability to improve the anti-oxidant capacities too.

Introduction

Aerobic organisms generate energy by reducing molecular oxygen to water. Biological molecules are oxidized during these processes generating reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulfur or free radicals (RS) [1]. Free radicals give rise to the oxidation of lipids, protein, DNA, as well as the inactivation of enzymes and membrane disruption. Aerobic organisms have been equipped with antioxidant system to limit the harmful effects of free radicals [1, 2]. The range of active antioxidants include: enzymatic antioxidants (superoxide dismutase (SOD) and catalase (CAT), glutathione peroxidase (GPx) and non enzymatic antioxidants (vitamin A, vitamin B, vitamin C), flavonoids, thiol glutathione (GSH), ubiquinone (Q_{10}), uric acid, bilirubin, ferritin and micronutrients (iron, copper, zinc, manganese). Oxidative stress is caused by an imbalance between the production of free radicals and in vivo antioxidant defense system. Damage in biological molecules is affected by this oxidative stress [2]. In a survey conducted by Ramel et al. the circular maximal strength test was used with 10 stations and 75% of (1 RM) one repetition maximum for 18 minutes. The findings have shown that the test gave rise to the increase in Malondialdehyde (MDA) levels after exercise in both trained groups (a group consisted of 7 men having experience in resistance activity and the other one consisted of 10 men having no experience in resistance activities but some other activities) stemmed from oxidative stress generated by exercise [5]. Similarly, Ficicilar et al. performed a study on animals having the mice run on a treadmill for half an hour within 3 consecutive days. They found that TAC level increased in mice after the exercise [6]. Another study conducted by Doris et al. on 9 masters of martial arts and 9 non-masters has shown that the modified Bruce test gave rise to the increase in GSH level of test group (masters of martial arts) compared to control group. Despite the beneficial effects of physical activity on health, many studies reported due to an increase in oxygen consumption and metabolic rate during exercise, the oxidative stress is generated by increasing the reactive oxygen species productions [8]. Anti-oxidative system maintains the cellular inner balance at rest and during the moderate exercise [9]. In long term and strenuous exercise, on the contrary, anti-oxidative defense is reduced due to increase in oxygen consumption and ROS production [1, 5, 10]. Eccentric exercise is a case in point. High-intensity eccentric exercise may lead to: cellular-muscle enzymatic increase in blood circulation, protoplasmic injury, inflammatory cell response to acute muscle injury, 24 to 48 hours after the exercise [11]. All these factors cause muscle enzymes to go into the blood stream, subsequently the oxidation of these enzymes takes place during physical activity. High concentration of these anti-

oxidative factors in the blood during the exercise prevented these enzymes from oxidation anyway. Since oxygen consumption greatly increases during the exercise which leads to increased free radical production, it must be noted that the eccentric-aerobic exercise is beneficial or detrimental for athletes knowing that it relates to high oxygen consumption and muscle injury. The reduced Glutathione (GSH), Malondialdehyde (MDA) and total anti-oxidation capacity (TAC) levels were measured in PE active women at Al-Zahra University before and immediately after the acute eccentric-aerobic exercise (before recovery) in order to determine the effect of acute exercise on oxidative and anti-oxidative systems.

Materials and Methods

Sixteen PE female students, Al-Zahra 2007 entries, were selected (mean age, average weight and average height were respectively: 20.85 yr, 54.46 kg, 5.35 feet or 163.37 cm). All PE students who entered Al-Zahra University in 2007 were gathered together. They were fully explained the survey. Thirty students volunteered to participate in this project. Sixteen students, among them, were selected randomly using pot method. They were divided into two groups of eight including control group and test group. They were all non-smokers taking no medicine especially contraceptive pills (birth control pills). They also didn't have any acute or chronic illnesses. All subjects including control group and test group had same breakfast, lunch and dinner 3 days before the exercise. They drank a cup of light brown tea for breakfast and ate two sugar cubes, 50 g of cheese, 50 g of walnuts and 100 g of bread as well 3 hours before the test. They did no physical activity for 48 hours prior the test. The subjects were asked to participate in a briefing meeting to be familiar with administration procedures as well as the purpose of study and there were also some points related to test the subjects needed to know. They were asked to do no severe physical activity at least 48 hours before the test. Resting heart rate was obtained from participations before the test and recorded in the relevant lists. Heart rate was measured with a polar heart rate monitor. Then Ellestad test was used in this regard.

The Ellestad test is the basic test for determining a person's aerobic fitness. It consists of seven stages: the first four periods take 3, 2, 2, 3 min respectively. The grade is 10% for the first four periods with treadmill speed at 1.7, 3, 4, 5 mph respectively. The last three periods each has 2 min duration and the grade constantly increases 15% in these periods. At the last three periods treadmill speed increases 6, 7, 8 mph respectively [13]. The subjects were asked to walk on reverse incline treadmill until exhaustion. (mean time to failure means the most exhaustive point when the subjects are unable to proceed the activity). Body mass index (BMI) was calculated by dividing the body weight (in kilograms) by the squared height (in meters) ($BMI=weight/height^2$). The blood samples were drawn from the subjects one hour before and immediately after the exercise. The blood samples were taken from the brachial vein of subjects in the sitting position kept in heparinized vacuum tubes (Venoject). Control group remained motionless (at rest position) during the experimental group activity. FRAP method was used to determine the plasma total anti-oxidative capacity (the sensitivity of method was 0.1 Units/ml). In this method, the antioxidants present in the sample give rise to the reduction of ferric tripyridyl triazine complex to ferrous form (in acidic environment changes to blue) which has the maximum absorbance at 593nm in the spectrometry. The reaction rate is linearly related to the strength reduction. Being limiting and strength reduction factor, Fe^{3+} used as an additional input in this way [14]. After the preparation of standard solutions and samples (EDTA 0.02 µ solution, tris buffer 0.04 µ containing 0.02 µ EDTA, 0.01 µ DTNB in methanol, GSH standard solution) a spectrophotometer at a wavelength of 412 nm was immediately used to measure samples and solutions absorption in order to determine the capacity of plasma reduced-glutathione, the GSH concentration in samples then calculated from the standard curve (the sensitivity of method was 0.5 micromole) [15]. Pouring 2.5 ml of tri-carboxylic acid solution TAC (10% w/v) into 0.5 ml plasma in a centrifuge tube, we put it in boiling water for 15 min in order to determine the MDA. After the tubes have become cool, we can centrifuge them at 1000 g for 10 minutes transferring 2 mm of its content to anther tube and adding 1 mm of thiobarbituric acid TBA (67% w/v) to it. Then the tube is to be placed in boiling water for 15 minutes. After the tube has become cool, its absorbance is read at 532 mm putting into equation $A=\varepsilon bc$. MDA then can be determined in this regard (the sensitivity of method was 0.8 micromole). The blood samples were drawn from the subjects one hour before and immediately after the exercise to determine the changes in blood oxidative and anti-oxidative parameters before recovering the body. Maintaining the cold chain during the transport, the samples (taken from the brachial vein of subjects) were transferred to Biomedical Research Laboratory of Al-Zahra College for women. Plasma was separated from blood cell, in vitro, using refrigerated centrifuge at 3000 rpm for 10 min at 40°C. To measure the GSH, MDA and TAC levels, plasma was stored in the freezer at -80˚C. Data derived from study, were processed using the statistical software (SPSS-13) and the inferential statistics were performed using a repeated measures analysis of variance (ANOVA). Quantitative data were expressed as mean±SD and significant difference at the level of $p<0.05$ was accepted.

Results

Data related to the profiles of female participants including height, weight, age, body mass index, mean time to failure and training intensity have been shown in table 1. The results derived from the measurement of plasma total antioxidant capacity, reduced glutathione and plasma malondialdehyde levels, before and immediately after the exercise, have been shown in graphs 1, 2, 3 respectively.

Table 1. Demographic characteristics of participants and training intensity

Variables Groups	N	Height (cm)	Weight (kg)	Age (yr)	Body mass index (BMI) (kg/m²)	Mean time to failure (min)	Training intensity (max HR)
Eccentric	8	163.05±2.17	55.62±1.38	21.37±0.49	20.82±0.49	9.19±0.45	88.87±1.38%
Control	8	161.87±2.58	55.50±1.96	20.52±0.18	21.15±0.43		

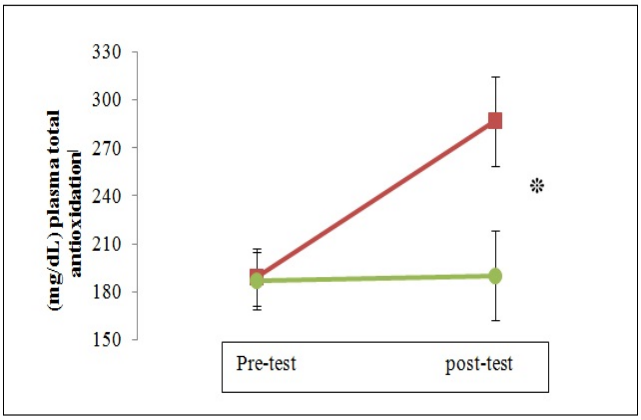

Figure 1. Comparing changes in the plasma total antioxidant level of eccentric and control groups over time, Red: eccentric, Green: control, *Significant effect of over time on p≤0.05 level

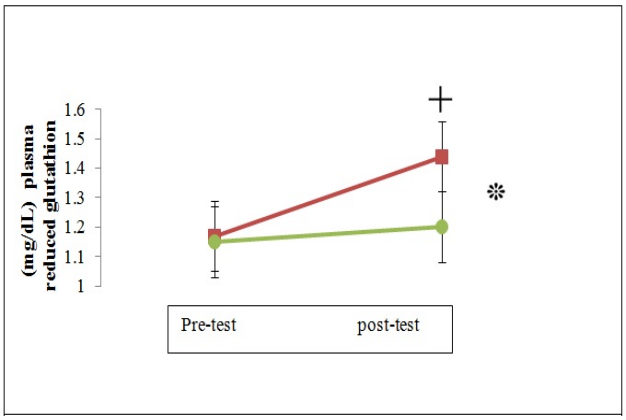

Figure 2. Comparing changes in the plasma reduced glutathione level of eccentric and control groups over time, Red: eccentric, Green: control,*Significant effect of over time on p≤0.01 level,+ Significant interaction between time and group on p≤0.05 level

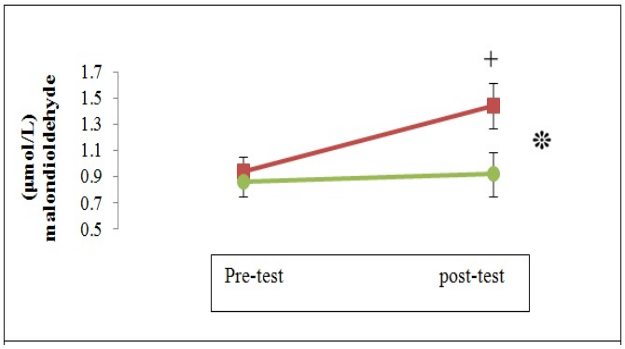

Figure 3. Comparing changes in the plasma MDA level of eccentric and control groups over time, Red: eccentric, Green: control, *significant effect of over time on p≤0.01 level, +significant interaction between time and group on p≤0.05 level

The plasma TAC level of eccentric group shown a significant increase in post-exercise compared to pre-exercise (p=0.033). However, the increase was not significant compared with the control group (Fig. 1). Plasma GSH level related to eccentric group, on the contrary, increased significantly after the exercise (p≤0.001). The increase was also significant compared with the control group (p=0.047) (Fig. 2). On the other hand, Plasma MDA level related to eccentric group shown significant increase (p≤0.001) which was also significant compared with the control group (p=0.04) (Fig. 3).

Discussion

The findings derived from present study shown that plasma reduced-glutathione level increased significantly after eccentric exercise compared to pre-eccentric exercise. The aforementioned increase was also significant after eccentric exercise in the experimental than in the control group. Eccentric contractions, as we know, can create skeletal muscle damage. Various parameters indicate the degree of muscle damage such as muscle soreness, movement extent, an increase in blood creatine kinase (CK) levels. This eccentric form of exercise, gives rise to the increase in blood creatine kinase enzyme. Having sulfhydryl residues, this enzyme can easily be oxidized which leads to decreased enzyme activity or enzyme deactivation. Since GSH prevents various enzymes from oxidizing caused by free radicals and reactive oxygen species, higher levels of GSH in blood plasma may be used to maintain the CK activity in the blood after the eccentric exercise against oxidative stress caused by reactive oxygen species produced as a result of subjects' activities [12].

Researches gathered over the past decade show that exercise creates an imbalance between ROS production and antioxidant defense due to the increased oxygen consumption which consequently causes oxidative stress and cellular damage in the body [17-20]. One of the most important antioxidant physiological systems in both human and animal is the glutathione antioxidant system using glutathione peroxidase to remove peroxides. GSH serves as a substrate for glutathione peroxidase [21]. In the present study, it has been observed that the amount of GSH level increased significantly after the exercise. This finding is consistent with earlier study conducted on 10 800-meter swimmers (aerobic) and 9 100-meter swimmers (anaerobic). In that study, it was observed that the plasma GSH levels increased 20 minutes after the exercise in both groups compared to pre-exercise due to

intensity training as well as the aerobic and anaerobic forms of metabolism generating free radicals [22].

The study conducted by Guillaume et al. on 6 healthy male desert marathon runners shown significant increase in plasma GSH levels after the exercise compared to pre-exercise.

The results were consistent with the present study. These findings, however, are inconsistent with following researches. In a study on trained and untrained men (22-57 years old), Kretzshmar et al. found that the plasma GSH levels decreased by 30% in trained group after the acute exercise on the ergo meter bicycle compared to pre-exercise, whereas the plasma GSH levels remained unchanged in the untrained group.

These results were similar to those of study by Laires et al. They suggested that the decrease in plasma GSH levels can be due to oxygen consumption in skeletal muscle which decreases the exit of GSH from muscle to plasma. The research conducted by Lee and colleagues also concluded that eccentric elbow exercise did not cause a significant change in blood GSH levels of young men after the exercise than the pre-exercise [26]. The probably factors related to the mechanism of increased GSH levels after the exercise are as follows. One of them is the activity of glutathione reeducates immediately after the exercise resulted in the recovery of GSH from GSSG in the present of NADPH. Hence, it can increase the plasma GSH levels in this way [27].

Another mechanism concerning increase in plasma GSH levels is hepatic glutathione during the exercise stemmed from the high concentration of glucagon and vasopressin levels in plasma. The reason why is that the GSH can be provided from amino acids (in food intake form) and endogenous amino acids by the liver. It is also able to get most of them into circulation. If exercise takes too long, the hepatic GSH storage will decrease led to decline in plasma GSH levels. The current research, however, includes short-term heavy eccentric exercise which has been led to increase in plasma GSH levels by liver (9 minutes and 19 seconds). In the present study it was also observed that the plasma TAC levels increased significantly after the eccentric exercise probably due to cellular damage caused by eccentric exercise, release of intracellular- muscle enzymes to the blood and high concentrations of GSH. These factors ultimately lead to plasma antioxidant concentration [11, 12].

This result (the increase in total antioxidant capacity of plasma after the exercise) was consistent with that of Child et al. They found that plasma total antioxidant capacity increased from 475±84 (mmol/1) to 564±11 (mmol/1) after the half-marathon in 17 male runners. The increase in plasma TAC levels is due to the increase in the levels of creatine kinase in the blood which consequently leads to high concentration of GSH as one of TAC factors [28]. These results were consistent with those of Subudhi et al. In this study, the plasma total antioxidant capacity increased 48 hours after the acute exercise in elite skiers. This result was attributed to some factors such as type, intensity, duration and frequency of exercise as well as the plasma central role in antioxidant [29].

There are many factors related to the increased plasma TAC level after the exercise. One of them is to restore antioxidants from tissue-to-plasma and contrast between different antioxidants which lead to plasma total antioxidant capacity improvement. Another factor is the increase in plasma GSH levels which has been proven right by recent study. Since plasma GSH is used as a determinate to evaluate the total capacity of plasma, the increased plasma TAC levels may have stemmed from the increase in plasma GSH levels [15]. Recent studies have shown that the physical activity can lead to increase in reactive oxygen species stemmed from the increase in oxygen consumption during the exercise. Among the reactive oxygen species are hydroxyl radical groups. They give rise to the lipid peroxidation. The MDA is their secondary products considered as indicators of oxidative stress [30].

In the present study it was observed that the plasma MDA levels increased significantly after eccentric exercise than before exercise. The increase was also significant in experimental rather than to control group. These findings have been confirmed in other studies. The results were also consistent with those of Goldfarb and colleagues conducted on eighteen women. In this study it was observed that the plasma MDA levels increased in both groups after the elbow eccentric exercise including experimental group who had taken the supplement and the control group who had not taken supplement. However, the increase was more significant for those who had not taken supplement. The increase of MDA in this study is due to poor performance of antioxidants leading to eccentric exercise-induced lipid oxidation [31].

These results were consistent with those of Shin and colleagues. Studying on 18 women including 8 experienced aerobic and 8 inexperienced aerobic women, they concluded that the increase in plasma MDA levels after the exercise in experienced aerobic group is due to lipid oxidation. These findings, however, are inconsistent with other studies. Heitkamp et al. concluded in a study on 30 women that the plasma MDA level decreased in experimental (experience with endurance exercise) than to control group (inexperience with endurance exercise) after 8 weeks of endurance exercise. The increase in plasma MDA levels after the eccentric exercise can be due to oxidative stress-induced lipid peroxidation. MDA is one of its main products having key role in the destruction of unsaturated fatty acids. The findings of this study indicate that acute eccentric exercise can cause significant changes in body's antioxidant system and produce oxidative responses. The increase in plasma total antioxidant levels and reduced-glutathione after the exercise shows the adaption of antioxidant system in this regard. The results of the present study confirm previous one concerning eccentric exercise (the increase in GSH, MDA, TAC levels). The increase in plasma MDA levels after the exercise (exercise-induced increase in MDA level) is probably due to the intensity of activity (high intensity) and lipid peroxidation induced by oxidative stress (increased production of hydroxyl radicals). In general, the anti-oxidative capacity improvement was

observed in the eccentric group in terms of duration and subjects' intensity training.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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