

Comparison of the Effect of Plyometric Training on Oxidative Stress and Biochemical Parameters among Tennis Players

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KEYWORDS Exercise. MDA. GSH. α -tocopherol. Oxidative

ABSTRACT The present research is aimed at examining the effect of 8-week plyometric trainings on oxidative stress and biochemical parameters among tennis players. In this study, 39 male certified male tennis players between the ages of 20-25 voluntarily participated. The participants in this research were divided into two groups randomly as control group (Age- 22.67 ± 1.61 , Height- 180.43 ± 6.85 , Weight- 77.43 ± 7.79 kg and BMI- 23.73 ± 1.23 kg/m²), and experimental group (Age- 22.76 ± 1.58 , Height- 176.40 ± 8.49 , Weight- 73.25 ± 10.04 and BMI- 23.45 ± 1.72). Experimental group were subjected to a program of 105-minutes; 35-minute plyometric training and 70-minute standard tennis training; while control group was engaged in 105-minute standard tennis training without any plyometric training for 8 weeks. Before and after 8-week training programs, blood samples were taken from the participants after 12-hour night fasting. Mann-Whitney U test was used for the analysis of pre-tests of groups; in-group pre-test and post-test differences were tested with Wilcoxon test. As a result, the findings showed that eight-week plyometric training increased MDA, GSH, and levels supports the assumption that regular physical activity has positive health effects.

INTRODUCTION

The impact of free radical as a reactive molecule of food into energy using oxygen is a process that has a lot of implications for the human body. Oxygen molecules are essential for life; and highly reactive intermediate products known as the resource of free radical occur during metabolism (Bagchi et al. 1998; Lamina et al. 2013). These molecules known as reactive oxygen types/metabolites damage cell components such as lipid, protein and DNA (Clarkson and Thompson 2000). Antioxidant defense systems are developed to keep free radical formation in aerobic (oxygen respiring) organisms and prevent the harmful effects of these molecules (Campos 2013). This system called antioxidant defense system includes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) enzymes and proteins such as transferrin, ferritin, and ceruloplasmin (Halliwell and Gutteridge 1995). However, existing antioxidant defense systems may not prevent the effects of free rad-

ical completely, and oxidative stress occurs (Matsuo and Kaneko 2000; Campos 2013).

Free radicals may occur in many ways such as exposition to UV rays, drugs, fat oxidation, immunologic reactions, radiation, stress, smoking, alcohol, and biochemical redox reactions. These free radicals may contribute to the occurrence of pathological cases including age-related degenerative disorders such as; atherosclerosis, heart diseases, cancer, cerebrovascular diseases, neurodegenerative diseases, diabetes, acute renal failure, lung diseases, emphysema, bronchitis, and alcoholic liver diseases (Bonney et al. 2002).

Exercise training resulted in physiological changes and adaptations that are highly beneficial to the human organism (Campos et al. 2012; Haskell et al. 2007; Nelson et al. 2007). However, high volume of training can also place a tremendous amount of stress on the organism and result in detrimental or mal-adaptations physiologically.

The researches have shown that free radical production increases especially during high intensity trainings and cause oxidative stress oriented cellular damages in various tissues (Inal et al. 2001; Manna et al. 2004). Studies that examine that effect of exercise on oxidative stress and antioxidant defense system mostly focus on aerobic exercises. These researches provide various findings. However, most of them report that

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long-term regular aerobic exercise reinforces antioxidant defense system and decreases the cellular damage caused by oxidative stress (Cazzola et al. 2003; Elosua et al. 2003; Ookawara et al. 2003; Fatouros et al. 2004). There are a limited number of studies on the subject, but these reports affirmed that anaerobic exercise causes oxidative changes in skeletal muscle and blood at macro-molecular level (McBride et al. 1998; Groussard et al. 2003).

The oxidative stress response to exercise is dependent on several factors including the intensity, duration, mode of exercise, and training status of the subject (Gomez-Cabrera et al. 2005; Hadzovic et al. 2014). However, most of the authors have focused only on the effects of endurance training on the responses and adaptations of antioxidant. In this regard, it is not clear whether the different modes of exercise can affect the oxidative stress response. More research is, however, needed on the influence of different mode of exercise and training on the oxidative stress response.

It is well recognized that acute strenuous exercise is accompanied by an increase in free-radical production and subsequent oxidative stress, in addition to changes in blood antioxidant status. Chronic exercise provides protection against exercise-induced oxidative stress by upregulating endogenous antioxidant defense systems. Little is known regarding the protective effect afforded by tennis exercise.

Plyometric training activities, involving a rapid stretch, followed by fast concentric contraction of the involved muscles, are commonly used by a wide range of athletes to increase jump performance and improve explosive power and muscular activation patterns (Ozbar et al. 2014). Majority of the research suggests plyometric training improves maximal strength performance and might reduce the risk of injury by enhancing functional joint stability in the lower extremities (O'Driscoll et al. 2011).

A large number of studies investigated the effects of plyometric training on bio-mechanical

and physical performance (Sáez de Villarreal et al. 2012; Turner et al. 2014a).

Based on the foregoing analysis of the inability of previous studies to determine changes in antioxidant responses after plyometric training plus tennis exercise, this study is therefore aimed at evaluating the effect of oxidative stress response of tennis players after eight week plyometric training.

MATERIAL AND METHODS

Subjects and Experimental Design

Based on the researchers quest to determine the effect of oxidative stress response of tennis players after eight week plyometric training, 9 male certified male tennis players between the ages of 20-25 voluntarily participated in the present research. Before their participation, they were provided with all the necessary information and they signed the volunteering certificate involving the work-plan and risks. Table 1 presents the training programs of control and experimental groups. During the research, participants were divided into two groups randomly as; experimental and control groups. Defining features of the participants are presented in Table 2. Experimental group conducted a program of 105-minutes; 35-minute plyometric training and 70-minute classic tennis training; while control group conducted a 105-minute classic tennis training without any plyometric training for 8

Table 2: Defining features of participants and statistical significance between experimental and control groups (Mann Whitney U test)

	<i>Exercise group (n=21) Mean±SD</i>	<i>Control group (n=18) Mean±SD</i>	<i>p</i>
Age (years)	22.76± 1.58	22.67± 1.61	0.886
Height (cm)	176.40± 8.49	180.43± 6.85	0.085
Weight (kg)	73.25±10.04	77.43± 7.79	0.114
BMI (kg/m ²)	23.45± 1.72	23.73± 1.23	0.704

Table 1: Training Program

Activity type: Plyometric training + Standard tennis training

Sets: 2 sets a day

Reps: 8 reps for lower extremity, 12 reps for upper extremity

Rest: 1 – 2 mins Intensity: 60-70% at maximum heart rate

Number of trainings-weekly: 3 days on Monday, Wednesday and Friday

Training duration of experimental group: 35 mins plyometric trainings and 70 mins standard tennis training

Training duration of control group: 105 mins standard tennis trainings

weeks. The present research is approved by Gaziosmanpasa University Commission of Scientific Researches Ethics Committee.

Anthropometric Measurements

Stature and body weight of the participants were measured with "Sport Expert™ Professional Sport Technologies" (Gerfan, Italy). Body weight was measured with a (kg) ± 0.01 kg sensitive bascule and height was measured with a (cm) ± 0.01 cm sensitive stadiometer; participants in anatomical posture, with standard sports outfit and without shoes before beginning resistance trainings.

Blood Samples and Analysis

Blood samples were taken twice; before and after 8-week training programs. 5 ml blood samples were taken from antecubital vein by an experienced staff after 12-hour night fasting in the morning, after participants were rested in lying position for about 10 minutes.

During taking of blood samples, ambient conditions were always the same and ambient temperature was always 18-20°C. Taken blood samples were directly delivered to Gaziosmanpasa University Central Laboratory in accordance with cold chain.

Parameters such as – Troponin, BUN, creatinine, ALT (alanine aminotransferase), AST (aspartate transaminase), and procalsitonin were examined using Roche diagnostics commercial kit (Manheim, Germany) with Roche Hitachi C501 autoanalyzer (Cobas 6000-Tokyo Japan) at Faculty of Medicine Biochemistry Lab.

Vitamins E and A were examined using RECIPE ClinRep brand Kit (Munich/ Germany) on SHIMADZU brand HPLC (High Performance Liquid Chromatography) device.

Malondialdehyde (MDA) levels that occur as the reason of lipid peroxidation of antioxidant enzymes; superoxide dismutase (SOD), peroxidase (GSH-Px) Protein carbonic (PC) and free-radicals and that indicate their damage to lipid structures were examined at spectrophotometer. Additionally, nitric oxide (NO) levels released from venous endothelium were examined.

The method of analysis used for antioxidant parameters examined in spectrophotometer:

SOD Activity Measurement: With the method developed by Sun et al. (1988) and modified

by Durak et al. (1993), it is based on regression of superoxide produced with xanthine/xanthine oxidase system to nitroblue tetrazolium (NBT). Absorbance of color formation was measured at 560 nm, and enzyme activity was detected.

GSH-Px Activity Measurement: In accordance with the method developed by Paglia et al. in the existence of hydrogen peroxide, GSH-Px catalyzes the oxidation of reduced glutathione to oxidized glutathione. During the oxidation of NADPH used in this reaction to NADP, absorbance reduction was measured at 340 nm, and enzyme activity was calculated (Paglia and Valentine 1967).

MDA Level Measurement: Malondialdehyde (MDA) and other TBARS create a pink colored chromogen when they react with thiobarbituric acid at 90-95°C. Samples were cooled quickly after fifteen minutes and their absorbance were read at 532 nm spectrophotometrically, and MDA levels were measured (Esterbauer and Cheeseman 1990).

NO Measurement: It was measured by measuring the color formed by the reaction of nitrite sulphanilamide produced with Griess reaction and modified cadmium reaction and NNDA diazotization at 545 nm spectrophotometrically (Cortas and Wakid 1990).

Protein Carbonic (PC) Levels: 2,4-Dinitrophenylhydrazine solution prepared in HCl was made to react with carbonyl compound, and precipitation washed with ethanol/ethyl acetate mixture 3 times, and after it dissolved in 100 mM NaOH solution, it was measured at 360 nm spectrophotometrically (Levine et al. 1990).

Data Analysis

Data analysis was conducted on IBM SPSS Statistics Version 20 packaged software. Defining data are presented as arithmetic average and standard deviation. To examine the effect of plyometric trainings, Wilcoxon signed-rank test was used in the statistical comparison of pre-training and post-training values of variables; and Mann Whitney-U test was used to compare the experimental and control groups. Significance level was taken as $p < 0.05$.

RESULTS

There are no significant differences ($p > 0.05$) between experimental and control groups in terms

of age averages (respectively 22.76 ± 1.58 and 22.67 ± 1.61 years), height averages (respectively 176.40 ± 8.49 and 180.43 ± 6.85 cm), weight averages (respectively 73.25 ± 10.04 and 77.43 ± 7.79 kg) and BMI averages (respectively 23.45 ± 1.72 and 23.73 ± 1.23 (kg/m²)) ($p > 0.05$) (Table 2). These indicate homogeneity among the two groups.

Further, there is no statistically significant difference between experimental and control groups in terms oxidative stress markers and biochemical parameters pre-test values (Mann Whitney U test; $p > 0.05$). This indicated homogeneity among the values of both groups.

This research also discovered that there are significant differences between pre-test and post-test SOD, MDA, GSH, Creatinine, Troponin and Vitamin E values of experimental group (Wilcoxon signed-rank test; $p < 0.05$), but no statistically significant changes were observed in other parameters (Wilcoxon signed-rank test; $p > 0.05$) (Table 3). Statistically, significant differences were found between pre-training and post-training BUN and Troponin values of control group (Wilcoxon signed-rank test; $p < 0.05$), while no statistically significant differences were observed for other parameters (Wilcoxon signed-rank test; $p > 0.05$) (Table 3).

DISCUSSION

To the best of the researcher's knowledge, this is the first study that seeks to evaluate the oxidative stress response to plyometric training in tennis players. The results of this study demonstrated that eight-week plyometric training in-

creased MDA, CSH, TRO and vitamin E levels and decreased SOD level.

GSH (γ -glutamylcysteinylglycine) is the most abundant nonprotein thiol source in the cell and serves multiple functions in protecting tissues from oxidative damage and keeping the intracellular environment in the reduced state (Turner et al. 2014b). The important role of GSH in protecting against exercise-induced oxidative stress has been reviewed in detail in several previous articles (Li Li 1999).

In the present research, it was observed that 8-week plyometric trainings with standard tennis trainings increased GSH levels. Among the current researches, there are some researches that found similar results with the present research (Sen et al. 1992; Leeuwenburgh et al. 1997; Karabulut et al. 2013; Bouzid et al. 2014); some other researches that found decrease (Leichtweis et al. 1997; Ramires et al. 1999); and some other researches that reported that there was no change in GSH values (Choi and Cho 2014).

Vitamin E (α -tocopherol), and β -carotene are important antioxidants that cannot be synthesized by many mammals and humans. Especially, vitamin E is very important during exercise for the prosecution of cellular activities. Rat with vitamin E deficiency demonstrated exacerbated muscle and liver free radical production and excessive lipid peroxidation and mitochondrial dysfunction after an acute bout of exhaustive exercise compared with vitamin E-adequate rats. Endurance performance has also been reported to decrease in rats fed vitamin E-deficient diets (Li Li 1999). The findings of the present research

Table 3: comparison of pre-training and post-training (8 Weeks) values of experimental and control groups (Wilcoxon signed-rank test)

	Experimental group					Control group				
	Pre mean \pm SD		Post mean \pm SD		p	Pre mean \pm SD		Post mean \pm SD		p
SOD	3.12 \pm	0.27	2.84 \pm	0.25	0.001	2.99 \pm	0.92	3.03 \pm	0.35	0.163
MDA	1.84 \pm	0.11	2.04 \pm	0.21	0.000	1.92 \pm	0.20	1.84 \pm	0.10	0.109
GSH	377.57 \pm	114.91	456.75 \pm	122.05	0.018	536.89 \pm	184.06	433.80 \pm	104.56	0.061
NO	127.26 \pm	7.17	122.42 \pm	6.47	0.255	129.57 \pm	11.42	127.58 \pm	13.70	0.717
PC	405.20 \pm	47.83	443.33 \pm	54.37	0.054	451.39 \pm	68.57	452.48 \pm	94.66	0.965
BUN	14.29 \pm	3.23	14.16 \pm	2.42	0.808	15.54 \pm	3.28	17.36 \pm	3.43	0.035
KRE	1.07 \pm	0.11	1.23 \pm	0.22	0.001	11.70 \pm	29.83	1.13 \pm	0.13	0.177
ALT	21.08 \pm	14.81	20.80 \pm	8.91	0.917	20.34 \pm	9.61	19.41 \pm	10.29	0.453
AST	31.49 \pm	27.78	27.59 \pm	9.48	0.768	25.71 \pm	8.91	23.23 \pm	4.81	0.155
TROPONIN	4.05 \pm	1.77	6.75 \pm	3.49	0.002	3.86 \pm	1.98	5.45 \pm	2.13	0.013
VitE	33.08 \pm	8.15	37.42 \pm	6.69	0.014	33.22 \pm	7.59	30.47 \pm	6.44	0.085
VitA	2.94 \pm	1.11	2.87 \pm	0.57	0.741	2.36 \pm	0.85	2.37 \pm	1.02	0.845

revealed that 8-week plyometric trainings with standard tennis training increased vitamin E, while no change was observed in vitamin A. There not many current researches on the effect of combined exercise type on vitamin levels, and they present contradictory results. The findings of these researches contradicted with the findings of the present research. Some of the current researches found decreasing in α -tocopherol as a result of acute and chronic exercises (Banerjee et al. 2003) while some others reported that there was no change (Secheck et al. 2003; El Abed et al. 2011; Bouzid et al. 2014). Possible reasons for these contradictions may be methodological differences such as; un-controlled diet, participant selection, intensity, duration, extent of exercise. Further researches are required in the subject field.

Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes over production of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress and the antioxidant status in exercise (Gawel et al. 2004). Regarding the effect of exercise on lipid peroxidation, our results showed a significant increase in MDA after 8 week plyometric training. These results are in agreement with the many work (El Abed et al. 2011; Bouzid et al. 2014; Hadzovic et al. 2014; Moghaddasi et al. 2014; Michalezyle et al. 2014), but are in disagreement with the findings of Karabulut et al. (2013) and Choi and Cho (2014). These conflicting results may be explained by the specificity of training loads applied to athletes and the physiological characteristics of subjects studied.

Exercise training may increase production of free radicals and reactive oxygen species in different ways. The training type and intensity may influence free radicals production, which leads to differences in oxidative stress status between athletes (Hadzovic et al. 2014).

CONCLUSION

This study is very important because, it is the first study that seeks to evaluate the oxidative stress response to plyometric training in tennis players. The results of this study demonstrate that eight-week plyometric training increased MDA, GSH, and α -tocopherol levels supports the assumption that regular physical activity has positive health effects in human organism.

RECOMMENDATIONS

It is suggested that regular physical activity and exercise are useful because it enhances the antioxidant defense system. Further well-controlled studies are recommended to clarify the relationship between the exercise intensity, exercise duration, mode of exercise on oxidative stress and long term health implications.

ACKNOWLEDGEMENTS

This work was supported and funded by the Scientific Research Projects Unit (2012/111) of the University of Gaziosmanpasa. The authors thank the institution which collaborated.

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