

The Effects of Progressive Relaxation Exercises Applied to Young Ski Jumpers on Oxidative DNA Damage

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ABSTRACT This research aimed to investigate the status of the reactive oxygen species, antioxidants, and oxidative DNA damage in the ski jumpers subject to progressive relaxation training. Experimental design was used in this research. Data was gathered from 12 male ski jumpers. Jacobson's PRT was applied to the athletes for 6 six weeks. Three blood samples were taken from the subjects. First, prior to the first session, second, immediately after the first session and third, immediately before the 4th session. According to the data, both short-term and long-term ski jumping exercises led to a significant increase in the MDA and NO levels that were only in the ski jumping group. Also, short-term and long-term training increased 8-OHdG values. The Relaxation Training with the ski jumping training sessions increased the MDA, NO and 8-OHdG values in term of... The biochemical condition that may occur with the implementation of progressive relaxation training with other exercises that can be considered intensive is interpreted in this research. Also, PRT suppressed the value of 8-OHdG with ski jumping exercises.

INTRODUCTION

Free radicals, occurring as a part of the metabolic processes in the cells, are mainly molecular components in the shape of oxygen or nitrogen containing more than one unpaired electron in their bonds (Halliwell and Gutteridge 2007). Reactive oxygen particles (ROS) can cause DNA damage and can lead to the formation of the harmful oxidative reactions when produced excessively as a free radical in the organism (Dal-Pizzol et al. 2001; Sonali and Madhusnata 2010). 8-OHdG is among the most typical oxidative DNA damage products (Jin et al. 2015). Antioxidant defense system functions for eliminating or reducing the impact of negative effects that may be created by free radicals through SOD, GPx, CAT in enzymatic structure and a variety of vitamins and minerals in non-enzymatic structure (Young and Woodside 2001). In the case of rise of the free radicals and exceeding capacity of the antioxidant system, oxidative stress arises (Bloomer and Goldfarb 2004). Free radical levels in the body in determining the oxidative stress, free radicals and DNA damage situation caused by lipid proteins and antioxidant activity status are used as a good biomarker (Stankovic and Radovanovic 2012). The excess of oxygen demand and use, generated by the means of exercise, also leads to an increase in the reactive oxygen particles (Martins et al. 2011; Powers et

al. 2011). Therefore, exercise is an oxidative stress tool leading to flux between oxygen particles and antioxidant defense system (Jackson et al. 2000). The intensity of the exercise is variable, which can affect the age and gender oxidative stress status beside the density, duration and exercise level. Reid (2000) and Pingitore et al. (2015) stated that exercises, with high density and strenuous ones inducing the overproduction of ROS, in contrast to the moderate exercise, were useful for oxidative stress. Ski Jumping is a sports branch, which contains the passion of height and is not considered ordinary in terms of the application area and is very popular among the winter sports. Sportsmen are required to have very good nerves muscle coordination, strength and balance in this branch that requires an excellent way of working mental, psychological and physiological systems in the body (Jost 2010). Researches on ski jumping are mostly concentrated on physics and aerodynamics, flight positions and postures of the athletes (Murakami et al. 2014; Jung et al. 2014). In this respect, investigation of this sport is physiologically and biochemically of great importance.

Developed in 1930 by Edmund Jacobson and used in different models by various scientists, Progressive Relaxation Exercise (PRT) is an exercise method involving voluntary contraction and relaxation of the large muscle groups. PRT was used as an alternative rehabilitation meth-

od to eliminate a number of different health problems including sleep disorders and the treatment of physical and psychological illness (Ghafari et al. 2009, Demiralp et al. 2010). The widespread use has become a subject in a lot of researches alongside.

Affecting the blood circulation and the system of the cells through contraction and relaxation, PRT is considered important to determine the effects on oxidative stress levels in ski jumping, a branch with high level of excitement. This research was conducted based on the hypothesis that Ski Jumping, whether is a sport that has enough intensity to induce the oxidative stress level of the athletes or not and whether oxidative stress levels are affected with PRT or not, is to determine the effects of the ski jumping and progressive relaxation exercises on oxidative stress.

METHODOLOGY

Subjects

A total of 12 male ski jumping athletes in the Turkish Ski Federation were randomly divided into experimental (SJ+ PRT group) and control groups (SJ group), with 6 athletes in each group. The experimental group had 14.67±2.73 age, 162.67±16.94 height, 49.33±13.52 weight and 18.26 ±1.60 BMI, and the control group had 13.50±2.74 age, 148.83±13.95 height, 37.17±11.05 weight and 16.32±1.56 BMI. All athletes who had no smoking history and were in good health participated in the experiment voluntarily and abided by a regular and rational diet during the experiment. Subjects were informed of the purpose of the study and the possible risks involved before giving their oral consent to participate.

Exercise Protocol

Working groups are formed as follows:

SJ (Ski Jumpers): Only athletes who practiced in ski jumping training. SJ group continued their practice only within its training program for 6 weeks.

SJ + PRT (Ski Jumpers + PRT): Athletes who practiced relaxation training in ski jumping.

The SJ + PRT group had applied progressive relaxation training protocol in ordinary training processes in addition to skipping trainings and just before the jump, the group was requested

to continue their regular training program along with the relaxation training protocol of the athletes in the 6 week period.

Jacobson's PRT was applied to the volunteers for six weeks (a total of 42 sessions; each session 20 minutes/day) as mentioned in the Guidebook (New Directions in Progressive Relaxation Training) (lit.). Three blood samples were taken from the subjects. First, before the first session (the control sample), second, immediately after the first session (the sample of acute PRT), and third, immediately before the 42nd session (the sample of chronic PRT), and the separated sera were stored at -80°C for the analyses of MDA, 8-OHdG, and NO levels and SOD and GPx activities.

The study protocol was in accordance with the Declaration of Helsinki and was approved by the local ethics committee (Ethical Committee of Atatürk University Health Sciences Institute).

Plasma MDA Measurement

Plasma MDA measurement was carried out by HPLC method defined by Khoschorur and coworkers (26). For this purpose, 50 µL of plasma was mixed with 750 µL of 0.44 mol/L H₃PO₄, 250 µL of 42 mmol/L thiobarbituric acid and 450 µL twice-distilled H₂O. The mixture was incubated in boiling water bath for 60 minutes. After incubation, the tubes were cooled in water-ice bath immediately. Alkaline methanol (1,5 ml) was added to each tube. The tubes were centrifuged at 3,000 rpm for 3 minutes, and 200 µL of supernatant of each tube was placed in a vial. RP-C18 (5µm, 4.6x150 mm) (EclipseVDB-C18; Agilent) column was used for determination. For making elution, a solution of ethanol plus phosphate buffer (50 mmol/L) at a ratio of 40:60 (v/v) was used. Flow rate was adjusted as 0.8 ml/min. From the samples prepared previously in vials, 20 µl was put in HPLC vials and placed on the machine. A FL detector determination was made by taking excitation at 527 nm and emission at 551 nm. MDA-TBA product peaks were calibrated by using 1,1,3,3-tetraethoxypropane standard treated similar to the samples. The results obtained were stated as µM.

DNA Isolation and Hydrolization

DNA isolation from the white blood cells in the whole blood was made with the method de-

finied by Adel et al. and Miller et al. (27, 28). The isolated DNA samples were stored at 20°C up to the analysis day. For the 8-OHdG analysis, the dissolved DNA samples were hydrolyzed with formic acid using Kaur and Halliwell's method (29). The hydrolyzed samples were dissolved in HPLC elution (final volume being 1 ml). EC detector was used for 8-OHdG determination in HPLC using 20 μ l of the final hydrolysate. A reverse phase C-18 (RP-C18) analytical column was used (250 mm \times 4.6 mm \times 4.0 μ m, Phenomenex, CA). A mixture of potassium phosphate (0.05 M; pH 5.5) and acetonitrile (97:3; v/v) was used as a mobile phase, and the flow rate was adjusted as 1 ml/min. The level of 8-OHdG was determined in ECD of HPLC adjusted to 600 mV (30) using a 8-OHdG standard (Sigma Aldrich Company).

GPx Analysis

GPx activity was measured by the coupled spectrophotometric assay at 340 nm from the oxidation of NADPH in the presence of H₂O₂ used as substrate (32). The principle of the test is as follows. GPx catalyzes the oxidation of glutathione in the presence of tert-butyl hydroperoxide (tBH). Oxidized glutathione was converted to the reduced form in the presence of glutathione reductase and NADPH, while NADPH was oxidized to NADP. Reduction in the absorbance of NADPH at 340 nm was measured. By measuring the absorbance change per minute and using the molar extinction coefficient of NADPH, GPx activity was calculated and expressed as IU/mL.

SOD Analysis

The SOD activity was detected according to the Sun and the coworkers (33). In this method, xanthine-xanthine oxidase complex produced superoxide radicals, which reacted with nitrobluetetrazolium (NBT) to form the farmasone compound. The SOD activity was measured at 560 nm by detecting the inhibition of this reaction. SOD activity was calculated and expressed as IU/mL.

Measurement Units were MDA and NO microM, SOD and GPx IU/mL, 8-OHdG pg/ml.

Statistical Analyses

Statistical analysis was conducted using SPSS 19.0. The data was not distributed symmetrically. Thus, K-Related Samples Friedman

test was used for the analysis of the repeated measurements and for the comparison of pre- and post-exercise data and chronic and acute mode data. In addition, 2-Related Samples Wilcoxon test was used in the evaluation of the groups as pairs. Mann-Whitney U test was conducted in the intergroup comparison measurement of the SSH and SSH + PORT group of rested, acute and chronic MDA, NO, SOD, GPX, and 8-OHdG. The results were evaluated on the basis of meaningfulness of 0.01 and 0.05.

RESULTS

In order to determine whether there is a difference between intra-group values of the Oxidative Stress markers SJ + PRT group SJ (MDA, NO, SOD, GPX and 8-OHdG) rested state values, Kruskal-Wallis tests were performed after the acute exercise and chronic training and the results were shown in Table 1.

Examined Table 1, it is observed that there was a significant relationship between MDA, NO, SOD, GPx and 8-OHdG values in the SJ groups ($\chi^2 = 12$, $P < .01$, $\chi^2 = 8.33$, $P < .05$, $\chi^2 = 9.33$, $p < .01$, $\chi^2 = 9.33$, $p < .01$, $\chi^2 = 10.33$, $P < .01$), ski jumping trainings increased significantly, and MDA, NO and 8-OHdG values both acutely and chronically, reducing the chronic SOD values, while they increase GPx values only chronically. Nevertheless, it was seen that there was a meaningful relationship between MDA, NO, SOD, GPx and 8-OHdG values of SJ + PRT groups ($\chi^2 = 12$, $p < .01$; $\chi^2 = 9.33$, $p < .01$; $\chi^2 = 7.00$, $p < .05$, $\chi^2 = 9.33$, $p < .01$, $\chi^2 = 10.33$, $p < .01$), relaxation trainings along with ski jumping increased significantly the MDA, NO and 8-OHdG values both acutely and chronically, reducing acutely while increasing chronically SOD values, they increase GPx values only chronically. Mann-Whitney U test was applied to determine whether there was a difference between the (MDA, NO, SOD, GPX and 8-OHdG) rested, acute and chronic groups of the oxidative stress level of the athletes in the control and experimental group and the results are shown in Table 2.

In Table 2 it was observed that there is a significant difference between the resting MDA values of SJ and SJ + PRT groups ($z = 2.882$, $p < .01$) and there was no significant difference in other parameters ($z = .048$, $p > .05$; $z = .32$, $p > .05$; $z = .32$, $p > .05$; $z = .16$, $p > .05$). Here it was observed that initial experimental and control groups of the athletes had similar levels of oxidative stress values and that there was a signif-

Table 1: Comparison of intra-group values of the rested SJ + PRT group SJ, acute and chronic MDA, NO, SOD, -group of GPx and 8-OHdG

Measurement	SJ (Just ski jumping training)				SJ+PRT (Ski jumping with prt)			
	N	Median	Chi-square	P	Median	Chi-square	P	Groups with difference
MDA	Rest	5.76	12	0.002	3.76	12	0.002	1-2* (.028)
	Acute	7.05			6.76			1-3* (.028)
NO	Rest	10.71	8.33	0.016	8.27	9.33	0.009	2-3* (.028)
	Acute	4.6			4.54			1-2* (.028)
SOD	Rest	5.21	9.33	0.009	5.21	7	0.03	1-2* (.046)
	Acute	7.23			8.64			2-3* (.028)
GPx	Rest	3.11	9.33	0.009	7.25	7	0.03	1-2* (.046)
	Acute	10.84			11.83			2-3* (.028)
8-OHdG	Rest	8.78	9.33	0.009	8.34	9.33	0.009	1-3* (.028)
	Acute	12.98			10.43			2-3* (.028)
	Rest	30.46	10.33	0.006	25.73	10.33	0.006	1-2* (.046)
	Acute	0.54			0.53			1-3* (.028)
	Rest	73			0.69			2-3* (.028)
	Acute	1.13			0.89			

* P < 0.05 ** P < 0.01

ificant difference between the 8-OHdG levels after acute training ($z = 2.402, p < .05$). No significant difference was found in other parameters ($z = .801, p > .05; z = .48, p > .05; z = 1.761, p > .05; z = 1.524, p > .05$), there was a significant difference between MDA and 8-OHdG values after chronic exercise ($z = 2.242; p < .05; z = 2.562; p < .05$), and no difference was encountered in other parameters ($z = 1.761, p > .05, z = 1.121, p > .05; z = .801, p > .05$).

DISCUSSION

This research was designed to determine the short-term acute (single session) and long-term chronic (the longer-term training) effects of the daily progressive relaxation trainings except the training programs of the young ski jumpers, on the oxidative stress level and antioxidant defense mechanisms. The researcher tried to explain what kind of impact progressive relaxation trainings provided on the physical and psychological relief along with ski jumping, which is an exciting sport caused on the level of oxidative stress in athletes.

Whilst scientific studies on relaxation training are usually conducted on anxiety and depression, Pender (1985) determined the effect of cognitive awareness and the effect of movement in the patients with circulatory disorder (Collins and Rice 1997), relaxation techniques in training science, performance in sports, anxiety, Weinberg and Comer (1994), concentration and motor skills were applied to determine the stress and arousal state (McCloy 1978). This study was aimed to determine to what extent progressive relaxation exercise, which is one kind of relaxation exercise, would impact the oxidative stress.

Exercise causes many physiological changes in the human body. These changes, which occur during the exercises, are based on the intensity, frequency and duration of the exercise (Sallam and Laher 2016). As one of the measures showing physiological response in exercises, antioxidant defenses and oxidative stress levels were studied for years. In this study, analogous pre-exercise oxidative stress levels of (SJ + PRT) experiment and control (SJ) groups indicated that the distributions of the groups were ideal (Table 2). According to the obtained data, it was seen that MDA increased significantly both the SJ+PRT and the SJ groups after the short-term (acute) and the long-term (chronic) exercises

Table 2: Comparison of intra-group values between SJ SJ+ PRT rested groups, MDA, NO, SOD, GPx and acute - chronic groups of 8-OHdG values

			REST			ACUTE			CHRONIC		
			Med- ian	Z	P	Med- ian	Z	P	Med- ian	Z	P
MDA	SJ	6	5.76	-2.882	.004**	7.05	-0.801	0.423	10.71	-2.242	.025*
	SJ+PRT	6	3.76			6.76			8.27		
NO	SJ	6	4.6	-0.48	0.631	5.21	-0.48	0.631	7.23	-1.761	0.078
	SJ+PRT	6	4.54			5.21			9.24		
SOD	SJ	6	8.13	-0.32	0.749	3.11	-1.761	0.078	10.84	-1.121	0.262
	SJ+PRT	6	8.64			7.25			11.83		
GPx	SJ	6	8.78	-0.32	0.749	12.98	-1.524	0.128	30.46	-0.801	0.423
	SJ+PRT	6	8.34			10.43			25.73		
8-OHdG	SJ	6	0.54	-0.16	0.873	0.73	-2.402	.016*	1.13	-2.562	.010**
	SJ+PRT	6	0.53			0.69			0.89		

* P <0.05 ** P <0.01

(Table 1). It was determined that after rested and chronic exercise of the SJ group, the MDA values were significantly higher than those of the SJ +PRT group, but no significant difference was reported after acute exercise (Table 2). It was found that "NO" levels increased significantly within both groups acutely and chronically (Table 1), and when compared to intergroup, no significant difference was found between them (Table 2). Oxygen consumption with exercise may increase 10-15 times (Banerjee et al. 2003). This excessive use of oxygen also constitutes an increase in the reactive oxygen particles (Martins et al. 2011; Powers et al. 2011). Ski jumping exercises as in a lot of other exercises led to an increase in the MDA and NO production. These results are consistent with the literature. While MDA levels were lower compared to those in the control group doing progressive relaxation exercise, the significant difference between intra-group MDA levels was found both before exercise and after acute and chronic exercise. It shows that the relaxation exercises did not impact positively the MDA levels of ski jumping athletes.

When the antioxidant defense mechanisms were studied, it was identified that the SOD values of the two groups increased acutely while reducing chronically (Table 1), and no significant difference was seen between SJ and SJ + PRT groups compared to the SOD levels of the intra-groups (Table 2). Observing the GPx values, it was detected that while no difference was seen between the acute values of the both groups (Table 2) they increased significantly

after the chronic exercises (Table 1), and no significant difference was found between SJ and the SJ + PRT groups compared to the GPx values between the groups after acute and chronic exercise, either (Table 2). Aerobic and anaerobic exercises, especially SOD and GPx, led to antioxidant substances to be induced. While Marzatico et al. (1997) and Knez et al. (2005) stated that training did lead to the reduction in the antioxidant capacity in which an increase was found in the antioxidant enzyme activity after an acute exercise, Cavas (2005) and studies were also reported where no change was seen (Vider et al. 2001). In this study, while the GPx values were similar to these studies, it was found that the SOD values decreased significantly, contrary to a lot of work in the literature as a result of the acute exercises. Watson et al. (2005) in a study that supported this situation pointed out that a reduction occurred in the antioxidant capacity after strenuous running exercises. A lot of work was reported in the direction of the long-term regular exercises affecting the antioxidant defense enzymes (Kabasakalis et al. 2009; Tonga 2013). It was stated that regular long-term exercises strengthened the antioxidant defense system in order to destroy or minimize oxidative stress case (Carlsohn et al. 2010) and provided physiological adaptation (Ørtenblad et al. 1997). Tongk et al. (2013) stated in his research that group of athletes doing long-term exercises had higher level of values compared to those of the control group in terms of antioxidant levels on professional young long-distance runners. Young cyclists, sedentary athletes and the rid-

ers had higher levels of values compared to those of the runners. It was seen that the study results on the antioxidant activity kept with the literature after the long-term exercise of studies.

It was observed that in both the SJ and SJ + PRT groups, intra-group 8-OHdG values significantly increased acutely and chronically (Table 1), while no significant difference was seen between the groups, relaxation exercises relatively suppressed the 8-OHdG increase resulting from the ski jump exercises (Table 2). The studies conducted on 8-OHdG were often on extensive and long-term durability studies (Reichhold et al. 2009). Okumura et al. (1997) in a study, conducted on 10 long-distance runners, found significant increases in the 8-OHdG values of the athletes after a daily training program. Oxidative stress increased depending on the intensity of exercise. This increase caused a rise in the 8-OHdG value, which was one of the best indicators of oxidative stress and deterioration in the mediated DNA mechanism of oxidative stress. However, adaptation to exercise and parallel to this development of antioxidant defense over time inhibited the growth. Sato et al. (2003) in their study found that the values of those sedentary were significantly higher as a result of a load of bicycle ergometer application for 30 minutes on 7 trained athletes and 8 sedentary ones. Allgayer et al. (2008) found significant reductions in the 8-OHdG value as a result of a moderate exercise that they performed on patients. Itoh et al. found a significant reduction in the 8-OHdG value after a running exercise. In this study, the rise of DNA damage showed that the ski jumping was a high-intensity exercise. The DNA damage, which was significantly higher but lower in the ski jumpers doing relaxation exercise, compared to those not doing exercise, suggested that in case of implementation of the longer-term relaxation exercises, they might suppress the oxidative stress.

CONCLUSION

According to the data obtained in this research, both short-term and long-term ski jumping exercises led to a significant increase in the MDA and NO levels only in the ski jumping group. While the SOD and GPx values were found to decrease post training, increases were detected as a result of the long-term training with GPx values. 8-OHdG values were also found to increase as a result of the short-term and long-term training.

In the group the researcher applied the relaxation training along with the ski jumping training sessions, it was observed that MDA, NO and 8-OHdG values increased significantly in both the acute and chronic values, reducing SOD levels acutely but increased chronically, while the GPx values increased chronically. Compared to these groups, although similar effects were seen, progressive relaxation training pressure significantly oppressed the 8-OHdG values.

RECOMMENDATIONS

Accordingly, biochemical conditions, which might emerge together with progressive relaxation training alongside with intensive-like exercises, might be interpreted. In order to better understand the physiological attributes of ski jumping, in terms of training science, more studies are needed on psychomotor and biochemical researches.

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