The Effect of Training Type on Oxidative DNA Damage and Antioxidant Capacity during Three-Dimensional Space Exercise

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Key Words

Three-dimensional space exercise • Free radicals • Oxidative stress

Abstract

Objective: Orbotron training is a popular training method for fighter pilots because it replicates a high-acceleration environment with excessive G-force. The purpose of this study was to investigate the effects of 9 weeks of orbotron training on oxidative DNA damage and antioxidant capacity in humans during 3-dimensional space exercise. **Subjects and Methods:** The subjects comprised 15 senior cadets from the Korea Air Force Academy who had no record of medical disorders and who participated in a regular exercise program (3 times per week). They were randomly divided into three groups consisting of 5 subjects each: a weight training group (21.97 ± 1.12 years), a running training group (21.53 ± 0.18 years) and an orbotron training group (21.48 ± 0.29 years). Three-dimensional exercise tests were performed before and after training, and blood samples were taken to measure the concentration of plasma lactate, malondialdehyde (MDA), erythrocyte superoxide dismutase (SOD) activity, and leukocyte DNA damage. **Results:** Plasma lactate concentrations decreased in all three groups when measured after training and after 30 min of recovery compared to before training ($p < 0.05$). The concentration of plasma MDA

also decreased after training in all blood samples compared to the values obtained before training although there was no significant difference in the weight training and orbotron training groups. In contrast, the activity of erythrocyte SOD increased for all three groups compared to before training ($p < 0.05$). In the comet assay results, the greatest lymphocyte DNA damage was demonstrated at the end of exercise compared to the other three samples under all conditions, and these aspects were commonly observed in all three parameters of lymphocyte DNA damage (tail DNA, tail length and tail moment) ($p < 0.05$). **Conclusion:** It can be concluded that the three types of exercise training reduced plasma lactate concentration, improved antioxidant enzyme activity, and further protected the body against oxidative stress (lipid peroxidation and DNA damage). Although we have identified an effect of exercise training on the levels of antioxidants and oxidants, our cohort was small, so further studies are needed to evaluate the different types of exercise training.

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Introduction

As air force pilots undertake missions in special 3-dimensional environments different from those on the ground, they experience physiological changes and oxi-

Table 1. Physical characteristics of the study subjects

Group	n	Age, years	Height, cm	Weight, kg	BMI	Body fat, %
WT	5	22.0 ± 1.1	174.7 ± 3.8	74.2 ± 8.7	24.3 ± 2.2	17.1 ± 4.1
RT	5	21.5 ± 0.2	175.0 ± 4.9	73.7 ± 4.9	24.0 ± 0.4	17.6 ± 1.3
OT	5	21.5 ± 0.3	173.3 ± 3.0	71.9 ± 12.8	23.9 ± 3.6	18.8 ± 7.1
F value		0.773	0.247	0.079	0.031	0.159
p value		0.483	0.785	0.925	0.970	0.855

Values are given as mean ± SD.

ductive stress from physical activities, with consequent damage which may be different from that experienced on the ground. During physical activities, the demand for oxygen by organs in the body increases and an excessive amount of oxygen is inhaled, resulting in an increased amount of oxygen free radicals and reactive oxygen species (ROS) which can hyperoxidate polyunsaturated fatty acid in cell membranes or damage protein and DNA, and thereby damage body functions [1]. However, the body has antioxidant mechanisms comprising various antioxidant enzymes to resist such toxic effects of oxygen free radicals and ROS, so it can ease their effects or get rid of them. Antioxidant nutrients can lower the concentration of oxygen free radicals and ROS or delay their impacts [2]. A number of reports have also shown that the intake of antioxidants decreased oxidative stress resulting from exercise in humans and rodents [3–5].

Researchers generally agree that severe exercise increases the production of oxygen free radicals and ROS and consequently causes damage to DNA in the body [6], but their opinions are inconsistent as to whether regular training activates antioxidant enzymes and protects the body from oxidative stress caused by exercise [7]. Moreover, research on oxidative stress caused by exercise and changes in the activity of antioxidant enzymes has been done mostly on the ground, and few studies have been undertaken in fighter pilots and astronauts who carry out their missions in special 3-dimensional aerial environments. Alterations to the antioxidant system induced by exposure to gravitational modulation are evidenced by the modification of poly(ADP-ribose) polymerase activity, which is strongly associated with the presence of DNA damage [8, 9].

In his experiment on space flight with rodents and humans, Stein [10] reported that oxidative damage increased after space flight. Different from the weightless space en-

vironment, air is a high-acceleration environment with excessive G-force, thus fighter pilots suffer from high physical stress, and their flights may result in the production of oxygen free radicals and ROS and consequent oxidative damage. Accordingly, not only for the individuals' health but also for national combat power, it is important to protect fighter pilots' bodies from oxidative stress during their operation in the aerial environment. However, there have been few studies in this special environment due to the difficulties in carrying out such research. It is therefore important to perform studies on these issues in the 3-dimensional aerial environment. Thus, the purpose of the present study was to use the comet assay to examine the effects of 9 weeks of regular training. It was conducted through three forms of exercise and assessed lipid peroxidation and DNA damage caused by oxidative stress that can take place in 3-dimensional space exercises that are most similar to activities in the aerial environment. In addition, this study attempted to determine whether aerial workers' regular exercise has any effect on protecting the body from oxidative stress and on maintaining homeostasis. It also aimed at analyzing differences between 3-dimensional space exercise and traditional aerobic-anaerobic exercise in overcoming oxidative stress caused by 3-dimensional space activities.

Subjects and Methods

Study Subjects

A total of 15 healthy Korea Air Force Academy cadets were selected for our study. They were randomly divided into three groups consisting of 5 subjects each – the weight training (WT) group, the running training (RT) group and the orobotron training (OT) group. To be included in our study, they had to be non-smokers and nondrinkers, and not to have been taking any vitamins. Informed consent was obtained from all study participants

before performing our experiment. The physical characteristics of the study subjects are displayed in table 1.

Physical Training

All experiments were performed from March 23rd, 2005 to June 10th of the same year. In the pretests, intensive exercise training for all participants was performed by voluntary constriction in the 3-dimensional space of an orbotron, and peripheral blood samples of each participant were collected in EDTA tubes 4 times before and after the experiments. Biochemical assay was performed on these blood samples to measure plasma lactate and malondialdehyde (MDA) concentrations, erythrocyte superoxide dismutase (SOD) activity, and lymphocyte DNA damage using the comet assay. After the pretests, members of each training group participated in a 9-week training program. The WT was performed by the circuit WT method at the level of 80% of one repetition maximum and repeated for 8–10 times per set for 3 sets per day. The RT consisted of running for 30 min per day at the level of 80% of maximum heart rate. The OT was controlled by the pace of 30 rotations per minute and the period of complete exercise was 15 min (5 sets of 3 min with 2–3 min rest between each set; 1 axis rotation for the first set with 2 min rest, 2 axis rotations for the second set with 2 min rest and 3 axis rotations for the third to fifth set with 3 min rest). After 9 weeks of training, posttests were performed in the same manner as for the pretests.

Biochemical Assay

Eight milliliters of blood were drawn from the peripheral veins of the subjects, placed in EDTA-containing tubes and immediately centrifuged for 10 min at 400 g in order to isolate the plasma. Plasma lactate concentration was measured enzymatically using an autoanalyzer (Kodak, EKTACHEM DT 60II), and the plasma MDA concentration was assayed spectrophotometrically by BIOXYTECH LPO-586 kit (Oxis, Co. Ltd., USA). Aliquots of whole blood were separated in heparin-containing tubes for erythrocyte SOD activity. The activity of erythrocyte SOD was determined in the supernatant of erythrocyte hemolysates. In brief, 1.0 ml of heparin-treated whole blood was mixed with 0.85% NaCl solution, centrifuged at 1,000 g for 10 min, the supernatant drained, and this step was repeated 4 times. Thereafter, the volume of this mixture was adjusted to 2.0 ml, incubated at 4°C for 15 min, and finally, analyzed by using an autoanalyzer (Cobas Mira, Roche, Switzerland).

Comet Assay

Measurement of DNA damage was performed using the comet assay according to the method of Anderson et al. [11] with slight modification. In brief, 260 µl of whole blood were mixed with 35 µl of 0.7% low-melting-point agarose on a glass slide, covered with a coverslip, and kept on ice for 5 min. Thereafter, the mixture was submerged in a lysing solution (0.1 M EDTA, 1% Triton X-100, 10% DMSO, 1.2 g/l Tris and 146.1 g/l NaCl), and the reaction was performed at 4°C for several days. Next, the slide was placed in an alkaline bath in order to denature DNA, and electrophoresis was performed for 40 min with a current of 300 mA and at 25 V. After electrophoresis, slides were treated 3 times with 0.4 M Tris buffer, and DNA staining was performed by the addition of 60 µl ethidium bromide (20 µg/ml). The comet measurement was shown by a computerized comet image, and it was recorded as tail length, percentage of DNA in tail and tail moment.

Statistical Analysis

All data were expressed as means $\pm$ SD. The paired t test was performed to analyze the difference between pre- and posttest values. One-way ANOVA was performed to compare biochemical parameters at rest, just after exercise, after 30 min of recovery, and after 24 h of recovery. Three-way ANOVA for repeated measurements was used to analyze the significant interaction effect among each training group (RT, WT or OT), each blood sample (rest, just after training, after 30 min of recovery or after 24 h of recovery) and each 3-dimensional test (pre- or posttest). Statistical significance was accepted at an α value of $p = 0.05$. All statistical analyses were performed using the SPSS for Windows version 12.0 package.

Results

Changes in Plasma Lactate, MDA Concentrations and Erythrocyte SOD Activity

The changes in plasma lactate, MDA concentrations and erythrocyte SOD activities before and after training are displayed in table 2 and figure 1. The plasma lactate concentration was decreased after training and after 30 min of recovery in all three groups compared to before training. A three-way ANOVA to analyze the interaction effect among each training group, blood samples and each 3-dimensional test for the determination of plasma lactate concentration revealed a significant effect for blood samples [$F(3, 36) = 196.569, p < 0.001$] and each 3-dimensional test [$F(1, 12) = 10.001, p < 0.05$]. The plasma MDA concentration decreased after training in all samples compared to before training. The RT group showed a statistically lower plasma MDA level at the end of exercise ($p < 0.05$) and after 30 min of recovery ($p < 0.05$) compared to before training. However, there were no statistically significant differences in blood samples taken at different times in the WT and OT groups. Among the three groups, RT showed the largest decrease in plasma MDA concentration while WT showed the smallest decrease. However, there were no statistically significant differences among all three groups under all of the experimental conditions. When a three-way ANOVA was performed, a significant effect was detected for each 3-dimensional test [$F(1, 12) = 20.009, p < 0.009$] and for blood samples [$F(3, 36) = 18.351, p < 0.001$]. The erythrocyte SOD activity increased in all blood samples taken after training compared to before training for all three groups. SOD activity increased at rest after training rather than before training for all groups. The RT group had a statistically higher level of SOD activity after 30 min of recovery ($p < 0.05$) compared to before training, and the OT group showed a higher level of SOD activity at rest ($p < 0.001$) and after 24 h of recovery ($p < 0.05$). No statistically significant differences were found in

Table 2. Changes in plasma lactate, MDA and erythrocyte SOD level following 3-dimensional space exercise before and after 9 weeks of training

Variable	Ex.	Treat	Before	After	Recovery (30 min)	Recovery (24 h)	F value
Lactate, mmol/l	WT	before	1.24 ± 0.28 ^a	8.04 ± 0.40 ^b	4.06 ± 0.22 ^c	1.30 ± 0.42 ^a	287.822**
		after	1.44 ± 0.58 ^a	6.68 ± 0.66 ^b	3.28 ± 0.48 ^c	1.44 ± 0.40 ^a	61.263**
		t value	-1.136	3.245*	3.509*	-0.455	
	RT	before	1.06 ± 0.11 ^a	7.50 ± 1.41 ^b	4.24 ± 1.09 ^c	1.14 ± 1.23 ^a	25.034**
		after	1.54 ± 0.49 ^a	5.58 ± 1.12 ^b	3.04 ± 0.33 ^c	1.50 ± 0.54 ^a	19.182**
		t value	-2.886*	3.430*	2.280	-0.859	
	OT	before	1.08 ± 0.30 ^a	7.94 ± 0.99 ^b	4.26 ± 0.29 ^c	1.34 ± 0.58 ^a	89.210**
		after	1.30 ± 0.62 ^a	6.88 ± 1.34 ^b	3.34 ± 0.56 ^c	1.68 ± 0.51 ^a	28.548**
		t value	-1.592	1.960	2.558	-1.753	
MDA, nmol/ml	WT	before	2.01 ± 0.27	3.06 ± 0.94 ^a	2.29 ± 0.58	1.73 ± 0.64 ^b	3.830*
		after	1.64 ± 0.28 ^a	2.60 ± 0.37 ^b	1.95 ± 0.36 ^a	1.53 ± 0.37 ^a	9.654*
		t value	1.823	1.346	1.527	0.753	
	RT	before	1.94 ± 0.19	3.16 ± 1.42	2.17 ± 0.75	1.84 ± 0.64	2.443
		after	1.58 ± 0.27 ^a	2.46 ± 0.91 ^b	1.75 ± 0.68 ^c	1.44 ± 0.29 ^a	2.876
		t value	2.295	2.880*	4.935*	1.044	
	OT	before	2.07 ± 0.35	3.24 ± 0.68 ^a	2.55 ± 0.91	1.81 ± 0.66 ^b	4.271*
		after	1.66 ± 0.73	2.59 ± 1.13	1.87 ± 0.44	1.57 ± 0.37	1.995
		t value	1.401	2.077	1.153	1.073	
SOD, U/g Hb	WT	before	612.4 ± 269.2 ^a	1,301.1 ± 597.7 ^{b, c}	773.8 ± 244.6 ^{a, c}	392.4 ± 204.5 ^a	5.639*
		after	676.7 ± 297.3 ^a	1,400.4 ± 575.6 ^{b, c}	765.9 ± 205.4 ^{a, c}	490.4 ± 297.9 ^a	5.669*
		t value	-1.744	-1.529	0.155	-1.870	
	RT	before	646.8 ± 234.1 ^a	1,373.9 ± 410.6 ^b	842.6 ± 177.4 ^a	558.6 ± 200.3 ^a	9.053*
		after	901.5 ± 305.6 ^a	1,608.2 ± 346.4 ^b	979.7 ± 145.5 ^a	654.4 ± 139.0 ^a	12.981*
		t value	-2.650	-2.375	-3.929*	-1.994	
	OT	before	676.5 ± 200.3 ^a	1,709.9 ± 559.8 ^{b, c}	1,107.1 ± 435.9 ^{a, c}	645.0 ± 243.2 ^a	8.201*
		after	912.6 ± 223.5 ^a	2,125.4 ± 877.1 ^{b, c}	1,329.8 ± 628.2 ^{a, c}	850.9 ± 321.1 ^a	5.233*
		t value	-12.461**	-2.364	-2.333	-4.484*	

Values are given as mean ± SD. * p < 0.05; ** p < 0.001. ^{a, b, c} Significant difference between letters (p < 0.05).

any of the blood samples from the WT group. There were no statistically significant differences among the three groups under any of the experimental conditions. The interaction analysis by three-way ANOVA indicated a significant main effect for each test [F(1, 12) = 30.496, p < 0.001] and for blood samples [F(3, 36) = 57.825, p < 0.001], respectively.

Changes in Lymphocyte Tail DNA, Tail Length and Tail Moment

The changes in lymphocyte tail DNA, tail length and tail moment before and after training are shown in table 3 and figure 2. The analysis of the comet assay to ex-

amine lymphocyte DNA damage indicated that the tail DNA was significantly higher at the end of exercise compared to the other three blood samples under all of the conditions, and decreased at all blood gathering times after training compared to before training in all three groups. The three-way ANOVA indicated significant effects for each test [F(1, 12) = 48.451, p < 0.001] and for blood samples [F(3, 36) = 321.079, p < 0.001]. The value of tail length was significantly higher at the end of exercise compared to the other three blood samples under all of the conditions, and decreased in all blood samples taken after training compared to before training in all three groups. The three-way ANOVA showed significant ef-

Fig. 1. Bar graph showing changes in plasma lactate (a), MDA (b) and erythrocyte SOD levels (c) before and after 9 weeks of OT.

fects for each test [$F(1, 12) = 113.264$, $p < 0.001$] and for blood samples [$F(3, 36) = 45.104$, $p < 0.001$]. In the case of tail moment, analyzed by tail DNA and tail length, this value was significantly higher at the end of exercise compared to the other three blood samples under all of the conditions, and decreased in all blood samples taken after training compared to before training in all three groups. This value significantly decreased when mea-

sured at rest, after 30 min of recovery, and after 24 h of recovery in the WT group ($p < 0.05$) and also decreased in all blood samples in the RT and OT groups ($p < 0.001$) (fig. 2). Like other parameters measured using the comet assay, the three-way ANOVA indicated significant effects for each test [$F(1, 12) = 95.405$, $p < 0.001$] and for blood samples [$F(3, 36) = 553.336$, $p < 0.001$], respectively.

Fig. 2. Bar graph showing changes in lymphocyte tail DNA (a), tail length (b) and tail moment (c) before and after 9 weeks of OT.

Discussion

This study was conducted with air force cadets and aimed to analyze the effects of three different forms of exercise (WT, RT, OT) on lipid peroxidation, erythrocyte SOD activity, and lymphocyte DNA damage. When the effects of 9 weeks of training on the concentration of plasma lactate through each form of exercise was evaluated, the WT group showed lower concentrations of plasma

lactate just after exercise and after 30 min of recovery after 9 weeks of training compared to values measured before training. On the other hand, the RT group showed a lower concentration of plasma lactate at rest before the 9 weeks of training and just after exercise after the 9 weeks of training. The OT group did not show any statistically significant difference among any of the blood samples taken at different times. In addition, all three groups showed the highest plasma lactate concentration just af-

Table 3. Changes in lymphocyte tail DNA (TD), tail length (TL) and tail moment (TM) following 3-dimensional space exercise before and after 9 weeks of training

Variable	Ex.	Treat	Before	After	Recovery (30 min)	Recovery (24 h)	F value
TD, %	WT	before	11.08 ± 1.47 ^a	20.01 ± 1.65 ^b	13.95 ± 2.53 ^a	12.40 ± 1.35 ^a	23.752**
		after	9.14 ± 0.62 ^a	19.10 ± 1.72 ^b	11.59 ± 1.10 ^a	9.39 ± 0.79 ^a	54.610**
		t value	2.863*	1.049	3.616*	4.411*	
	RT	before	10.91 ± 1.72 ^a	19.93 ± 2.28 ^b	12.87 ± 1.45 ^a	11.38 ± 1.17 ^a	30.259*
		after	8.92 ± 1.28 ^a	17.38 ± 1.99 ^b	10.33 ± 1.18 ^a	9.35 ± 1.24 ^a	36.874**
		t value	6.325*	11.290**	5.585*	4.340*	
	OT	before	11.05 ± 1.99 ^a	19.42 ± 2.34 ^b	12.20 ± 1.62 ^a	11.69 ± 1.65 ^a	20.753**
		after	9.22 ± 0.73 ^a	18.44 ± 1.68 ^b	10.67 ± 1.34 ^a	9.52 ± 1.68 ^a	47.820**
		t value	2.590**	1.917	2.282	4.466*	
TL, μm	WT	before	41.98 ± 5.55 ^a	58.74 ± 4.88 ^b	44.78 ± 5.64 ^a	43.17 ± 6.10 ^a	9.841*
		after	38.07 ± 3.40 ^a	55.86 ± 3.00 ^b	41.89 ± 5.50 ^a	39.28 ± 3.97 ^a	20.264**
		t value	2.971*	2.681	3.130*	3.227*	
	RT	before	42.21 ± 4.85 ^a	58.67 ± 5.90 ^{b, c}	49.94 ± 3.58 ^{a, c}	44.37 ± 5.02 ^a	11.194**
		after	37.74 ± 2.67 ^a	50.59 ± 5.00 ^b	39.96 ± 3.38 ^a	39.00 ± 2.47 ^a	14.092**
		t value	3.384*	6.115*	43.748**	3.504*	
	OT	before	40.52 ± 3.53 ^a	60.43 ± 2.83 ^b	46.22 ± 3.60 ^a	42.53 ± 4.00 ^a	32.694**
		after	38.36 ± 3.22 ^a	55.19 ± 4.37 ^b	43.29 ± 3.49 ^a	40.27 ± 3.10 ^a	22.233**
		t value	4.634*	6.601*	4.481*	3.078*	
TM, %	WT	before	3.59 ± 0.34 ^a	10.70 ± 0.40 ^b	5.18 ± 0.88 ^a	5.11 ± 1.87 ^a	42.850**
		after	2.48 ± 0.44 ^a	9.66 ± 1.06 ^b	3.82 ± 0.83 ^a	2.69 ± 0.51 ^a	100.983**
		t value	4.848*	1.825	5.250*	2.715	
	RT	before	3.57 ± 0.65 ^a	10.70 ± 1.82 ^b	5.42 ± 0.80 ^a	4.01 ± 0.51 ^a	46.072**
		after	2.35 ± 0.39	7.73 ± 0.80	3.12 ± 0.48	2.63 ± 0.41	1.803
		t value	7.138*	6.340*	9.855*	7.872*	
	OT	before	3.44 ± 0.71 ^a	10.72 ± 1.37 ^b	4.64 ± 0.88 ^a	3.95 ± 0.62 ^a	65.032**
		after	2.53 ± 0.36 ^a	9.16 ± 1.14 ^b	3.62 ± 0.72 ^a	2.84 ± 0.77 ^a	76.214**
		t value	3.542*	3.445*	3.260*	6.023*	

Values are given as mean ± SD. * p < 0.05; ** p < 0.001. ^{a, b, c} Significant difference between letters (p < 0.05).

ter exercise compared to the other three blood samples (at rest, after 30 min of recovery, and after 24 h of recovery). In addition, despite the increase in the concentration of plasma lactate when measured at rest after the 9 weeks of training, all three groups showed a lower plasma lactate concentration just after exercise and after 30 min of recovery after the 9 weeks of training compared to the value measured before training. Specifically, just after exercise, the concentration decreased by 16.5% in the WT group, 25.7% in the RT group and 21.6% in the OT group. These results suggest that if physical strength increases through training, the intensity of exercise felt by the body becomes lower for the same load of exercise, and as a result less lactate is produced. From this viewpoint, the

three types of exercise applied in this study were all effective. In addition, the reason for the larger decrease in the RT group compared to the other groups may be due to metabolic adjustments caused by aerobic training such as the increase of enzymes involved in aerobic metabolism and the increase of blood flow that took place as a way of decreasing the accumulation of lactate. The results of analyzing the effects of 9 weeks of training on the concentration of plasma MDA caused by oxidative stress in each form of exercise showed that all three groups demonstrated a tendency for the concentration to decrease in all blood samples taken after training compared to the values measured before training. After the 9 weeks of training, the WT group showed an 18.4% decrease when mea-

sured at rest, 15.0% just after exercise, 14.5% after 30 min of recovery and 11.6% after 24 h of recovery; the RT group had 18.6, 22.2, 19.4 and 21.7% decreases, respectively, and the OT group 19.8, 20.0, 26.7 and 13.3% decreases, respectively. Moreover, in comparison within the groups, just after exercise after the 9 weeks of training, the WT and RT groups showed increases in the concentration of plasma MDA ($p < 0.05$), but values in the OT group remained unchanged compared to the measurement at rest ($p > 0.05$). Therefore, our data support previous reports that training of the same intensity decreased the concentration of plasma MDA [12, 13]. In addition, our data suggest that training under the same environmental conditions decreased the concentration of plasma MDA in special 3-dimensional environmental exercise.

In general, intense exercise can increase oxidative stress and consequently result in various tissue damages including lipid, protein and DNA. The major cause of oxidative damage is attack by ROS, and ROS has little clinical significance. In their special 3-dimensional environment, air force pilots would experience much more oxidative stress than those on the ground. However, because it is dismutated by the antioxidant defense system, SOD is present in tissue to convert O_2^- to H_2O_2 and H_2O_2 to H_2O and O_2 [14]. Deficient or inactive SOD enzyme elevates O_2^- levels in intact blood vessels. In this study, SOD activity significantly increased after training in the OT group. Some reports on the efficacy of endurance training to antioxidant enzyme activity consistently suggest that regular endurance exercise training and OT stimulate cytosolic and mitochondrial antioxidant enzyme activity [7, 15–18] with a greater increase in the mitochondria fraction [19, 20].

Lymphocyte DNA tail length and DNA tail moment are biomarkers for DNA oxidative damage in humans. DNA damage increases remarkably in human serum after exercise, as reported previously [21]. When the comet assay was performed in this study in order to see the effects of the 9 weeks of training on lymphocyte DNA damage according to the form of exercise under all experimental conditions, the damage level was statistically higher just after exercise than in all three blood samples taken at different times, and these three parameters were similar in all three groups studied. This shows that the three types of training applied in this study decrease lymphocyte DNA damage. Statistically, the WT group showed significant decreases in all three parameters at rest, after 30 min of recovery and after 24 h of recovery during the 9 weeks of training, and the RT group showed significant decreases for all three parameters in all blood samples

taken after the 9 weeks of training. In addition, the OT group showed a significant decrease in tail DNA when measured after 24 h of recovery and significant decreases in tail length and tail moment in all blood samples taken after the 9 weeks of training. According to the literature, a bout of exercise is considered to cause oxidative stress and to increase DNA damage [22], but it is still controversial as to what effects long-term training has on DNA damage in the human body. Although it is considered more likely that regular training decreases DNA damage [23–25], there are also reports that it failed to decrease DNA damage [26, 27].

DNA damage depends on antioxidant defense capacity because the postexercise DNA damage level in people with higher antioxidant protection capacity was 60% lower than in subjects with lower antioxidant protection capacity [28]. As a result, low lymphocyte DNA damages in the RT and OT groups are caused at least in part by the high SOD activity levels due to aerobic training and/or OT. As discussed above, the explanation for these results is that regular aerobic and/or orobotron exercise increased the activity of erythrocyte SOD against oxidative stress and therefore strengthened the body's resistance and homeostasis to overcome harmful effects resulting from the production of ROS. Accordingly, regular aerobic and/or orobotron exercise is considered to produce beneficial effects for the body to adjust itself to repeated oxidative stress.

Conclusion

The three types of exercise executed during 9 weeks of training in this study resulted in a decrease in the concentration of plasma lactate through 3-dimensional space exercise. In addition, it was found that 9 weeks of training with three types of exercise increased the activity of antioxidant enzyme and consequently decreased the concentration of plasma MDA and lymphocyte DNA damage. With regard to the effect of 3-dimensional space exercise on overcoming oxidative stress, no statistically significant difference was observed in the effect of 9 weeks of training between traditional aerobic-anaerobic exercise and 3-dimensional space exercise.

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