

The Effects of Detraining in Young Elite Wrestlers: Malondialdehyde, Total Oxidant Status, Total Antioxidant Status, Glutathione

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ABSTRACT The aim of the present study was to investigate the effects of a 12-week detraining period after a 9-month multicomponent wrestling training program on total antioxidant status (TAS), glutathione (GSH), malondialdehyde (MDA) and total oxidant status (TOS) and some markers of performance. Fourteen young wrestlers (age: 14.9±1.1 years) and twelve non-athletes (14.5±0.5 years), making a total of twenty-six, participated in the study. MDA and TOS were significantly higher during competition period in wrestlers compared to control ($p < 0.05$). GSH was significantly higher in control group compared to wrestlers during competition period. There was no significant difference in TAS between controls and wrestlers during the competition period ($p > 0.05$). The results of this study indicate that strenuous exercise leads to an increase in the production of MDA and TOS in wrestlers. Despite the detraining period, MDA and TOS concentrations were still higher in wrestlers compared to the control group.

INTRODUCTION

Elite wrestlers generally participate in strenuous exercise during the preparatory and competition season. During the season, the wrestlers encounter substantial physiological stress via aerobic and anaerobic training, weight loss, caloric restriction, practice, competition, and potential injury throughout the course of the competitive season (Ratamess et al. 2013). However, many athletes often experience interruptions while training and competition process due to illness, injury, post-season break or other factors, which may result in reduction of their habitual physical activity level (Mujika and Padilla 2000). Because of these reasons, depending on the duration of insufficient training stimuli, training-induced adaptations reduce. Four week post-season break is considered short term, while longer than that period is referred to as long term (Mujika and Padilla 2000).

Regular exercise training induces an adaptation within the body, which relates to an up regulation of antioxidant protection mechanisms

(Miyazaki et al. 2001). Antioxidant defense system serves to defend against future increased free-radical attack (Finaud et al. 2006; ElAbed et al. 2011). Under normal physiological conditions, there is a balance between endogenous oxidants and various antioxidant defenses. The excessive generation of oxidants or a decrease of antioxidants results in an imbalance is called oxidative stress (Yanagawa et al. 2010). Regular exercise is likely to produce a positive effect on antioxidant and oxidative damage repair systems (Radak et al. 2002; Radak et al. 2008). The most often used methods for estimating oxidative stress are measurements of free radical-mediated damage to lipids (lipid hydroperoxides as primary oxidation products and malondialdehyde (MDA) as a secondary oxidation product (Dopsaj et al. 2013).

Young athletes generally take 3 months off from the regular training after their last major competition. The purpose of the present study was to examine how a 12-week detraining period could affect malondialdehyde (MDA), glutathione (GSH), total oxidant status (TOS), total antioxidant status (TAS) and some markers of performance as aerobic endurance and hand grip strength, after a 9-months multicomponent training program in young wrestlers. Based on the above, this study attempted to address these questions by comparing antioxidant and oxidative stress status during the period of time between the peak of in-season (after a 9-months

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multicomponent training program) and off-season (a 12-week detraining period) and to compare this with the data obtained from the healthy men in the control group. It was hypothesized that the training and the detraining periods may have deleterious effects on markers of some of oxidant and antioxidant in athletes. The aim of this study is to verify the presence and eventually the magnitude of some of the biochemical changes in elite young wrestlers after a period of long-term training and detraining.

METHODOLOGY

Subjects

Young male wrestlers (n=14, age: 14.9±1.1 years, height: 1.57±0.1 cm, weight: 46.6±9.9 kg) who were selected from Wrestling Education Centre in Çorum, Turkey, participated as experimental group in the present study. The control group (n=12, age: 14.5±0.5 years, height: 1.57±0.1 cm, weight: 51.1±8.4 kg) consisted of participants with a sedentary lifestyle. The consent of the subjects were obtained, even in written form, both in groups and from their parents, before testing. The study complied with the Declaration of Helsinki, and was approved by the Bioethics Commission of the University of Kirikkale.

Training Protocol

The wrestlers participated in training programs of 1.5-hour exercise in a day and 6 days per week during nine months. The nine months training season was divided into four training parts. *The first period* lasted for two months and was meant for basic preparatory consisting of aerobic, strength, and technique – tactic exercises with an average of 70-85 percent HR max for all active tasks. *The second period* lasted for four months and was based on general preparatory which consists; aerobic, strength, technique – tactic, coordination and balance exercises with an average of 80-90 percent HR max for all active tasks. While *the third period* lasted just only one month and it was on specific preparatory and was based on aerobic-anaerobic power, strength, technique – tactic, coordination, balance, reaction, and wrestling workout exercises with an average of 90 -100 percent HR max for all active tasks. And *- the last period* was for two months based on competition term of technic

and tactical training, preparation match and official match with an average of 90 -100 percent HR max for all active tasks. The competition period was followed by a detraining period of three months.

Testing Procedures

Blood samples were taken from the wrestlers during both the competition period (after the 9 months) and detraining period (after the 3 months from the competition period). However, Control group blood samples were taken during the midterm (in the middle of the period between the competition and detraining period). The blood samples were collected at the same time from each wrestler and control group. All samples were taken in the morning just after the wake-up and before breakfast. The samples were prepared as appropriate and stored at -80°C until biochemical analyses were performed. The performance tests were performed at the same time during both the competition (after the 9 months) and detraining periods (after the 3 months from the competition period). The control group did not practice any exercise activity. Due to this inactivity requirement, the aerobic endurance and hand grip strength performance tests were not performed by the control group.

Measurements of Height and Body Weight

Body height and weight measurements were collected with participants in bare feet and wearing only shorts. Height was measured to the nearest 0.1 cm and body mass was measured to the nearest 0.1 kg using a calibrated scale (Seca 714, Hamburg, Germany).

Maximal Isometric Hand Grip Strength

Hand grip strength was measured for dominant hand with a Dynamometer (Takei A5001 Hand Grip Dynamometer Tokyo, Japan). The subjects were placed sitting with 0° of shoulder flexion, 90° of elbow flexion and the forearm in neutral position. Two trials were performed and the best score of two measurements was recorded.

Aerobic Endurance Test

Aerobic endurance was determined by using a shuttle run multistage (20 meter) test. And

prediction of VO₂max was made using Leger and Gadoury's equation (1989). The initial speed was 8.0 km/h which got progressively faster (0.5 km/h. every minute) in accordance with a pace dictated by a sound signal on an audiotape. The wrestlers were instructed to keep pace with the signal for as long as possible.

Measurement of Malondialdehyde (MDA)

Marker of Lipid damage: MDA

For the determination of MDA levels, a derivatization step was used, in which protein-bound MDA was hydrolyzed (60 min at 95°C) and converted into a fluorescent probe. The fluorescent probe was cooled to 2-8°C, centrifuged at 10,000 g for 5 min, mixed with a reaction solution and injected into the HPLC system (Agilent Technologies 1200 series, USA; kits: Immuchrom GmbH, Germany). The isocratic separation via HPLC at 30°C, (Bischoff ProntoSIL Eurobond, 5 μm, 125 mm x 4 mm; Germany), lasted for 4 min for each sample, using a reversed-phase column. The chromatograms were recorded by a fluorescence detector. Quantification was performed with the calibrator from the kit. The concentration was calculated via integration of the peak heights using the following equation:

$$\text{conc}_{\text{sample}} = (\text{peak height}_{\text{patient}} \times \text{conc}_{\text{calibrator}}) / \text{peak height}_{\text{calibrator}}$$

Measurement of Glutathione (GSH)

The levels of GSH were measured. During the derivatization reaction, glutathione was converted into a fluorescent probe. A subsequent precipitation step removed high molecular weight substances. After centrifugation at 10,000 g for 5 min, the fluorescent probe was cooled to 2-8°C and injected into the HPLC system. The isocratic separation via HPLC at 30°C was performed with a reversed-phase column (MZ Inertsil ODS, 5 μm, 125 mm x 4 mm) in 2 runs. Each of these runs lasted 4 min. The chromatograms were recorded by a fluorescence detector. The quantification was performed with the delivered EDTA-blood calibrator; the concentration was calculated using the internal standard (IS) method. GSH was calculated by the following equation:

$$\text{conc}_{\text{sample}} = [(\text{peak area}_{\text{patient}} \times \text{conc}_{\text{calibrator}}) / \text{peak area}_{\text{IS patient}}] \times F$$

$$F = \text{peak area}_{\text{IS of the calibrator}} / \text{peak area}_{\text{calibrator}}$$

Measurement of Total Antioxidant Status (TAS)

Serum TAS was determined using a novel automated colorimetric measurement method developed by Erel (2004). In this method, a hydroxyl radical is produced by the Fenton reaction and reacts with the colorless substrate *o*-dianisidine to produce the bright yellowish-brown dianisyl radical. The results were reported as micromol Trolox equivalents per liter (μmol TroloxEq/L).

Measurement of Total Oxidant Status (TOS)

Serum TOS was determined using a novel automated colorimetric measurement method developed by Erel (2005). In this method, the oxidants that were present in the sample oxidized the ferrous ion substrate *o*-dianisidine complex to the ferric ion. This reaction was enhanced by the glycerol molecules present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in the acidic medium. Subsequently, the color intensity, related to the total amount of the oxidant molecules, was spectrophotometrically measured (Biochrom Libra). This assay was calibrated with hydrogen peroxide, and the results were reported as μmol H₂O₂Eq/L.

Statistical Analysis

The data were analyzed with analysis of variance (ANOVA) and post hoc least significant difference (LSD) tests using the SPSS version 22.0 statistical software package (SPSS Inc., USA). A probability level of $p < 0.05$ was used as an index of statistical significance. The standard deviation of the mean is indicated along with the mean. To decide whether the data follow the normal frequency distribution or not, it may be sufficient enough to examine the coefficients of skewness and kurtosis (Paz-Gonzalez et al. 2000).

RESULTS

Some of the physical profiles in control group and wrestlers were presented in Table 1. While malondialdehyde, total oxidant status, total antioxidant status, glutathione, hemoglobin and hematocrit parameters in control group and wrestlers were presented in Table 2. And aerobic endurance and hand grip strength values in wrestlers - were presented in Table 3.

Table 1: Some of the physical profiles in control group and wrestlers

Variables	A (n=12)	B (n=14)	C (n=14)	p
Age (year)	14.5±0.5	14.9±1.1	14.9±1.1	NS
Height (cm)	1.57±0.1	1.57±0.1	1.60±0.1	NS
Weight (kg)	51.1±8.4	46.6±9.9	49.4±9.6	NS
BMI	20.6±3.0	18.7±2.3	19.1±2.0	NS

p >0.05 NS: not significant A: Control group; B: Competition period for wrestlers; C: Detraining period for wrestlers

Table 2: The comparison of malondialdehyde, total oxidant status, total antioxidant status, glutathione, hemoglobin, hematocrit between the wrestlers and control group

Variables	A (n=12)	B (n=14)	C (n=14)	p
MDA (imol/l)	1.35± 0.23	2.40± 0.36	1.96± 0.08	A-B, A-C, B-C
GSH (imol/l)	342.7± 82.3	264.5± 89.1	186.2± 17.6	A-B, A-C, B-C
TAS (µmolTroloxEq/L)	2.15± 0.56	2.39± 0.47	1.97± 0.26	B-C
TOS (µmol H ₂ O ₂ Eq/L)	2.04± 0.42	2.78± 0.57	2.35± 0.33	A-B, B-C
HGB (g/dL)	13.4± 1.2	15.0± 1.3	14.2± 1.4	A-B
HCT (%)	37.7± 3.2	41.9± 3.9	40.8± 4.1	A-B, A-C

*p<0.05, A:Controls; B: Competition period for wrestlers; C:Detraining period for wrestlers, MDA: malondialdehyde; GSH: glutathione; TAS: total antioxidant status; TOS: total oxidant status; HGB: Hemoglobin; HCT: hematocrit

Table 3: The comparison of aerobic endurance and hand grip strength performances in wrestlers after 9-month training period and after 12-week detraining period

	Competition period (n=14)	Detraining period (n=14)	p	Dif. %
Aerobic endurance (ml.kg.min ⁻¹)	53.5 ± 2.7*	48.2 ± 2.8*	0.00	-9.9
Strength (kg)	37.0 ± 7.9*	32.0 ± 7.9*	0.00	-13.5

Significant level: *p<0.05 Difference: Dif.

There is no any significant differences (age, height, weight, BMI) between the control group and wrestlers (p>0.05) (Table 1).

MDA and TOS were -77.7 percent and 36.3 percent significantly higher respectively during peak training session in wrestlers compared to control (p< 0.05; Table 2). However, GSH was 29.6 percent significantly higher in control group compared to wrestlers during the peak training session. There was no significant difference in TAS between controls and wrestlers during competition period (p>0.05; Table 2). MDA, GSH, TAS and TOS were 18.3 percent, 29.6 percent, 17.6 percent and 15.5 percent significantly lower respectively in wrestlers after detraining period (p< 0.05; Table 2). MDA was also 45.2 percent significantly higher in wrestlers when compared with the control after detraining. GSH was 84 percent significantly lower in wrestlers when compared

with the control group after detraining period (p< 0.05; Table 2). Hemoglobin and hematocrit status were 10.6 percent and 10 percent significantly higher respectively during peak training session in wrestlers compared to control (p < 0.05; Table 2).

Aerobic endurance (VO₂max) and hand grip strength performance values were significantly decreased (53.50±2.7- 48.2±2.8 ml.kg.min⁻¹, 37.0±7.9 - 32.0±7.9 kg respectively) between the competition period and detraining period in wrestlers (p < 0.05) (Table 3).

DISCUSSION

The findings of the study provide, for the first time, a reasonable information about serum oxidant (MDA and TOS) and antioxidant status (TAS and GSH) regarding the training and detraining periods in adolescent wrestlers. The main

objective of the study was to investigate the TOS (the total amount of oxidant molecules), concentrations of MDA (Marker of Lipid damage), TAS, which includes the radical hydroxyl, and the levels of GSH in elite young wrestlers, during the detraining period and to compare these with the data obtained from the healthy men in the control group. To our knowledge, no study has investigated the effects of training and detraining on the concentration of MDA, GSH, TAS, TOS, HGB and HCT during off season.

The study revealed that resting concentrations of MDA, TOS were remarkably 77.7 - 36.3 percent higher respectively - in wrestlers during the peak training season in basal status than those of the sedentary group ($p < 0.05$) (Table 2). However, resting concentrations of TAS was not significantly different, but relatively 11.2 percent higher in wrestlers during the peak training season in basal status than those of sedentary group (Table 2). Yet, GSH was significantly 29.6 percent lower in wrestlers group compared to the sedentary group during peak training session (Table 2). Similar results have been observed in the literatures studies. El Abed et al. (2011) reported that resting concentrations of MDA were higher in the group of judokas than - that of sedentary subjects and concentrations of MDA increased significantly for 80 percent of participants. The results of the study - show that concentrations of TAS recorded at rest and after exercise were significantly higher in the judokas compared to in the sedentary subjects (El Abed et al. 2011). This result reflects an adaptation of the antioxidant system under the training effects of exercise. In a similar study, Gougoura et al. (2007) found that child swimmers exhibited 25 percent higher oxidative stress at basal state than their untrained counterparts and TAC was found lower by 28 percent in swimmers compared to the controls. Ugras (2012) reported that MDA levels increased significantly and some antioxidant enzyme activities (catalase: CAT, superoxide dismutase: SOD) were decreased in the period of post training and after the championship when compared to the pre-training period for both female and male Muay Thai athletes. There is an agreement with results of this study with previous studies. However, some differences emanated from the results found in the literature. Sharifi et al. (2014) found that MDA level in the female handball athlete group (regular handball training 3 times a week for at least 6 months) was

lower than in the non-athlete group. Brites et al. (1999), total plasma antioxidant capacity was 25 percent significantly higher in soccer players than in sedentary controls. This study (Brites et al. 1999) revealed that soccer players, under regular training, show an improved plasma antioxidant status in comparison to sedentary subjects. The researchers concluded that regular exercise training may increase the activity of antioxidant enzymes and reduces oxidant production and markers of lipids peroxidation like MDA. El Abed et al. (2011) found that, concentrations of TAS recorded at rest and after exercise were significantly higher in the judokas compared to the sedentary subjects. Azizbeigi et al. (2014) reported that endurance, resistance and concurrent training, consisting of 3 times/week on nonconsecutive days for 8 weeks, significantly decreased resting MDA levels by 32.7- 32 and 28 percent respectively. However, TAC increased significantly in the endurance and concurrent training groups compared with the pretest values. The researchers (Azizbeigi et al. 2014) concluded that the decrease of MDA resting level was caused either by the increase in antioxidants activity or by the decrease in the production rate of free radicals and an associated decrease in oxidative stress. Yamaner (2010) reported that the TAS of the soccer players (trained at least three days a week, 2 h daily) was higher than that of the control group. Hamurcu et al. (2010) found that regular wrestling exercise in adolescents may improve antioxidant enzyme Paraoxonase-1 (PON1), Nitric oxide (NO) level, and can be beneficial to inhibit oxidative DNA damage. These results demonstrate that, regular wrestling exercise to reduce oxidative damage may cause positive adaptations for antioxidant defense mechanism. The researchers (Hamurcu et al. 2010) reported that regular wrestling exercise for a healthy life is important since it reduces DNA damage while enhancing antioxidant parameters. Tromm et al. (2012) suggest that, exercise sessions at least 3 times a week for eight weeks are necessary to reduce oxidative damage and to increase the activity of antioxidant enzymes in the liver and in the heart of animals. These conflicting results may be explained by the type of sport, duration of training period, and the specificity of training loads applied to athletes and the physiological characteristics of subjects studied. Balci et al. (2010) reported that, various forms of exercise result in oxidative stress of different se-

verities. In addition, the study also revealed that, there were significant differences in the level of hemoglobin (HGB) and hematocrit (HCT) between the wrestlers and control group, which were 10.6 percent and 10 percent respectively higher in the wrestlers (Table 2). The regular exercise and training loads applied to athletes may result into increase in HGB and HCT levels.

The results emanating from this study show that, in wrestlers, concentrations of MDA, GSH, TAS and TOS were significantly low with 18.3 - 29.6 - 17.6 and 15.5 percent respectively in the detraining period than peak-exercise period (Table 2). However, the study established that, MDA was still 45.2 percent significantly higher and TOS was relatively 15.2 percent higher in wrestlers after detraining at basal state than their untrained counterparts (Table 2). In addition, GSH was 84 percent significantly low in wrestlers compared with the controls after detraining (Table 2). Such a finding may indicate that, the intensive and regular training loads may lead to such a discrepancy in athletes compared to their counterparts. The study and previous studies indicate that, differences in exercise intensity training frequency and durations (hour) in a week could play an important role in oxidant/ antioxidant parameters.

The results from the study also showed that, a 12-week detraining period abolished some of physiological benefits induced by multicomponent training program (Table 3). One of the main findings is that, VO_{2max} ($ml \cdot min^{-1} \cdot kg^{-1}$) and hand grip strength (kg) values decreased under peak values after the 12-week detraining. The decrement in VO_{2max} and hand grip strength was 9.9 and 13.5 percent respectively after detraining (Table 3). Oliveira et al. (2009) reported that, after 40 days of detraining a statistically significant decrease in performance in the 20m running test and a decrease in the maximum strength in the squat was observed. Ormsbee and Arciero (2012) reported that, after 5 weeks detraining, competitive collegiate swimmers body weight (training: 68.9 ± 9.7 kg, detraining: 69.8 ± 9.8 kg), fat mass (training: 14.7 ± 7.6 kg, detraining: 16.5 ± 7.4 kg), and waist circumference (training: 72.7 ± 3.1 cm, detraining: 73.8 ± 3.6 cm) increased significantly, whereas VO_{2peak} (training: 46.7 ± 10.8 $ml \cdot kg \cdot min^{-1}$, detraining: 43.1 ± 10.3 $ml \cdot kg \cdot min^{-1}$) decreased significantly. Another study reported that, 5 weeks of reduced training (RT) or training cessation (TC) resulted significantly into a greater decline in 1RM strength (-8.9 and -7.8 percent, $p < 0.05$, respectively, for bench press (BP) and

prone bench pull (PBP)) than those observed for RT (-3.9 and -3.4 percent) and declines in maximal aerobic power were lower for RT -5.6 percent than for TC -11.3 percent (García-Pallarés et al. 2010). The study and literatures' results illustrate the need to maintain aerobic and muscle strengthening exercise training in athletes during the off season to help prevent decreasing aerobic endurance and strength due to long detraining periods.

CONCLUSION

The results of the study indicate that, wrestlers have higher concentrations of TOS and MDA as compared to sedentary subjects. Despite the detraining period, MDA and TOS concentrations were still higher in wrestlers compared to the control group. This is likely to be the result of their strenuous exercise, which can potentially overwhelm antioxidant defense and lead to oxidative stress. The study also indicates that, the long detraining period decreases aerobic capacity and muscle strengthening in athletes. And off season training may include non-tiring aerobic and muscle strengthening exercise to protect the achievements that obtain all training season.

RECOMMENDATIONS

The researcher to this study hereby recommends that future or further and more research should be carried out on the effects of detraining on this parameter as it addresses the need to further experiment and clarify the TAS, TOS, MDA, GSH response to detraining.

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