Effect of Wrestling Exercise on Oxidative DNA Damage, Nitric Oxide Level and Paraoxonase Activity in Adolescent Boys

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The objective of the current study is to determine the effects of regular wrestling exercise oxidative DNA damage and antioxidant parameters. The findings of the current study have shown that 8-hydroxy-2’-deoxyguanosine (8-OHdG) obtained from wrestlers in basal status were significantly lower than those of sedentary \( (p = .001) \). In contrast, Nitric oxide (NO) and Paraoxonase-1 (PON1) were remarkably higher in wrestlers in basal status than those of sedentary (respectively, \( p = .001 \), \( p = .024 \)). While the NO of wrestlers increased immediately after a 1.5-h exercise compared with those before exercise \( (p = .002) \), no differences were found between before and immediately after a 1.5-h exercise in 8-OHdG and PON1 (respectively, \( p = .777 \), \( p = .408 \)). Statistically significant correlations were found between the NO and PON1 in the wrestlers in basal status \( (r = .671, p = .002) \). In conclusion, our study suggests that wrestling exercise for a healthy life is important in that it reduces DNA damage as well as enhancing antioxidant parameters.

Regular physical exercise is known to be beneficial to health; it improves cardiovascular function, vascular tone, muscular strength, endurance \( (6,9,29,31) \). However, the energy demand during physical exercise causes an increased oxygen uptake, which may increase the production of reactive oxygen species \( (2,7,31,32) \). Therefore, it has been claimed that some types of prolonged physical exercise are detrimental to health.

Oxidative DNA damage may occur if the increase in ROS concentration exceeds the protective capacity of antioxidant defense mechanisms \( (2,19,29,31) \). 8-hydroxy-2’-deoxyguanosine (8-OHdG) is one of the damaged DNA products caused by reactive oxygen species \( (21,32) \). 8-OHdG can be measured with high sensitivity and it is extensively investigated in human and animal exercise studies \( (21) \). While exercise increased 8-OHdG level in some studies \( (1,29,32,41) \), no change of 8-OHdG level was reported in others studies \( (7,15,16,26) \). It is suggested that the discrepancy in results may be attributed to the difference in the intensity of training of the subject, exercise types, and duration \( (26,29) \).

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Endothelial dysfunction is one of the risk factors for cardiovascular disease (14). It is suggested that exercise improves vascular endothelial function (39). Nitric oxide (NO) acts as a vasodilator produced by endothelial cells, and exercise has an important impact on the bioavailability of NO (34). Despite the available information on exercise-dependent bioactivity of NO, little is known about the effects of exercise on either endothelial function or oxidative stress (22,34).

Formation of free radicals during exercise is neutralized by an increase in antioxidant defense mechanism (8,12). Paraoxonase-1 (PON1) is an antioxidant HDL-linked enzyme, and its activity is influenced by both genetic and environmental factors (12). It is suggested that physical exercise is associated with PON1 activity. The effect of exercise on PON1 activity has been studied in few, and the results are contradictory. While exercise increased PON activity in some studies (8,12), no change of PON activity was reported in other studies (23).

Regular exercise is likely to produce a positive effect on antioxidant and oxidative damage repair systems (31,34). The adaptive effects of regular exercise depend on the characteristics of exercise (34). Wrestling exercise is an intense exercise that involves both aerobic and anaerobic exercise (27). To the best of our knowledge, the effect of regular wrestling exercise on oxidative damage has not been investigated as yet in wrestlers. The major objective of the current study is to determine and compare 8-OHdG level, NO level and PON1 activity in the sera of adolescent male wrestlers who has been on regular exercise for six months and of the sedentary individuals serving as the control group. A secondary objective is to investigate serum 8-OHdG, NO levels and PON1 activity in wrestlers before and immediately after a 1.5-hr wrestling exercise.

**Materials and Methods**

**Subject**

Eighteen adolescent male wrestlers who were selected from Talas/Kayseri, Turkey. Wrestling Education Centre and 18 adolescent male control subjects were studied. The wrestlers participated in training programs of 1.5-hr exercise per day, 6 days per week during six months. The training was coached by 1 of the wrestling team coaches. The training program consisted of a mix of aerobic, endurance-type training, wrestling skill enhancement, and strength training exercise (27). The training consisted of the following: Warm-up (20 min): Jogging and stretch exercise, Technique drills (20 min): The participations performed typical wrestling skills, Situation wrestling (15 min): The participants were paired. Specific wrestling positions were assigned, and participants wrestled from the given situation practicing a specific move and its counters, Iron man (15 min): Wrestlers were placed in groups of 5 or 6 with 1 wrestler in each group designated the iron man. The iron man continuously wrestled facing a new partner every 30 s. Designation of iron man rotated after approximately 3–4 min. Live wrestling (10 min): Each wrestler was paired with a partner of similar weight and ability. Each pair wrestled a full 6-min match. None of the participants trained during the day preceding the blood sampling. The control group was formed of students with a sedentary lifestyle with no practice of exercise activity.

The wrestlers and controls were not placed on any special diet, and no subject was taking any vitamins, mineral or other supplementation. The mean age and body
mass index (BMI) of wrestlers and controls were not statistically different (Table 1). The mean age and BMI of wrestlers and controls appear to belong to the same population according to age and BMI compared with the data obtained from each group.

Informed consent was obtained from all subjects before the study. The study protocol and the procedures were approved by the local ethical committee. The study was conducted in accordance with the Declaration of Helsinki or local laws depending on whichever afforded greater protection to the subjects.

**Samples**

In basal status and immediately after a 1.5 hr exercise, blood samples were drawn into plain vacutainer tubes from wrestlers. Also, blood sample were collected in plain vacutainer tubes from controls. All sampling procedures were performed at the same time, place and in the same conditions (i.e., fasting). Blood samples for analysis were immediately centrifuged at 3000 rpm for 15 min at room temperature. All serum samples were then stored in microtubes at -80 °C until assay for 8-OHdG level, NO level and PON1 activity.

**Measurements**

**Determination of 8-OHdG Levels.** The 8-OHdG in serum was measured using an ELISA kit (Catalogue: NWK-8OHDG02, Northwest Life Science Specialties). Serum 8-OHdG levels were expressed in ng/mL. Calibration, curve fitting and data analysis were done according to the instructions of the manufacturer.

**Determination of NO Levels.** Serum NO was measured using Nitrate/Nitrite Colorimetric Assay Kit (Catalogue: 780001, Cayman Chemical, and Ann Arbor, USA). Serum NO levels were expressed in µM. Calibration, curve fitting and data analysis was done according to the instructions of the manufacturer.

**Determination of PON1 Activity.** Serum PON1 activity was measured according to a method described elsewhere (3,11). We measured the rate of hydrolysis of paraoxon by monitoring the increase of absorbance at 405 nm and at 25 °C. The basal assay mixture included 1.0 mM paraoxon and 1.0 mM CaCl₂ in 0.05 M glycine buffer pH 10.5. One unit (IU) of paraoxonase activity is defined as 1 mol of p-nitro phenol formed per min, and activity was expressed as U/L of serum. All chemicals used in this study were from Sigma Chemical Co. (St. Louis, MO, USA) and were of analytic grade or highest grade available.

**Statistical Analysis.** Differences between data obtained from wrestlers and control subjects were investigated using the nonparametric Mann–Whitney U-test for two independent samples. Differences before exercise and immediately after exercise in wrestlers were investigated using nonparametric Wilcoxon test for two related samples test. The significance level was set at $p < .05$. Spearman’s rho correlation analysis was used to assess the relationships among 8-OHdG level, NO level and PON1 activity.

**Results**

The characteristics of subjects in both groups were shown in Table 1. There was no statistically significant difference of age, weight, height and BMI values between the groups ($p > .05$, Table 1).
The means of 8-OHdG levels from wrestlers and controls were shown in Figure 1. The 8-OHdG levels obtained from wrestlers in basal status were significantly lower than those of control subjects ($p = .001$, Table 2). However, no difference was found between the wrestlers’ basal status and immediately after exercise in 8-OHdG level ($p = .777$, Table 2).

The NO levels and PON1 activity were remarkably higher in wrestlers in basal status than those of controls (respectively, $p = .001$, $p = .024$, Table 2). While the

Table 1  Characteristics of Control and Wrestler Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age (years; Mean ± SD)</th>
<th>Weight (kg; Mean ± SD)</th>
<th>Height (cm; Mean ± SD)</th>
<th>BMI (kg/m²; Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 18)</td>
<td>14.11 ± 0.68</td>
<td>52.72 ± 11.74</td>
<td>161.83 ± 10.21</td>
<td>20.81 ± 5.30</td>
</tr>
<tr>
<td>Wrestler (n = 18)</td>
<td>13.89 ± 0.95</td>
<td>50.19 ± 11.88</td>
<td>159.28 ± 10.53</td>
<td>20.08 ± 4.04</td>
</tr>
</tbody>
</table>

$p = 0.152$  $z = 1.526$
$p = 0.181$  $z = 1.364$
$p = 0.214$  $z = 1.268$
$p = 0.767$  $z = 0.316$

Table 1 Notes: SD = Standard Deviation, BMI = Body Mass Index.

Figure 1 — Means of 8-OHdG levels from serum of wrestlers and controls. Abbreviations: BE: Before exercise, AE: Immediately after exercise.
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NO levels of the wrestlers increased immediately after a 1.5 hr exercise compared with those in basal status ($p = .002$), no difference was found between the wrestlers basal status and immediately after exercise in PON 1 activity ($p = .408$, Table 2).

Statistically significant correlations were found between the NO level and PON activity in the wrestlers in basal status ($r = .671$, $p = .002$).

**Discussion**

The benefit of regular exercise (nonexhaustive physical exercise) in promoting good health and preventing various diseases has been well known for a long time. However, exhaustive exercise also represents a form of oxidative stress to organisms and, therefore, can alter the balance between oxidants and antioxidants (9). The beneficial effect of regular exercise, at least in part, is due to oxidative stress induced adaptation (7,25,33). It is reported that the oxidative adaptive process of exercise is probably dependent on the increase in antioxidant level and oxidative damage repair enzyme (33,34).

8-OHdG is one the most abundant base modifiers which is formed by reaction of hydroxyl radical at the C-8 position of the guanine on DNA (10,17). The modified bases may cause miscoding, mutation and synthesis of a variety of incorrectlyed proteins (15,30,41). The upregulation of 8-OHdG repair is an important process because of the mutagenic potential (35). It is reported that the activity of 8-oxoG

<table>
<thead>
<tr>
<th>Group</th>
<th>NO (µM) (Mean ± SD)</th>
<th>PON (U/L) (Mean ± SD)</th>
<th>8-OHdG (ng/mL) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Controls (n = 18)</td>
<td>6.34 ± 1.88</td>
<td>274.74 ± 87.95</td>
<td>0.43 ± 0.16</td>
</tr>
<tr>
<td>Wrestlers (Before exercise; n = 18)</td>
<td>11.79 ± 3.73</td>
<td>334.89 ± 124.93</td>
<td>0.30 ± 0.03</td>
</tr>
<tr>
<td>p=</td>
<td>0.001*</td>
<td>0.024*</td>
<td>0.001*</td>
</tr>
<tr>
<td>z=</td>
<td>4.129</td>
<td>2.274</td>
<td>3.593</td>
</tr>
<tr>
<td>B Before exercise (n = 18)</td>
<td>11.79 ± 3.73</td>
<td>334.89 ± 124.93</td>
<td>0.30 ± 0.03</td>
</tr>
<tr>
<td>Immediately after exercise (n = 18)</td>
<td>15.94 ± 5.09</td>
<td>312.21 ± 169.64</td>
<td>0.30 ± 0.06</td>
</tr>
<tr>
<td>p=</td>
<td>0.002*</td>
<td>0.408</td>
<td>0.777</td>
</tr>
<tr>
<td>z=</td>
<td>3.114</td>
<td>0.828</td>
<td>0.283</td>
</tr>
</tbody>
</table>

*p < .05

Note. A: Controls and wrestlers (basal status), B: Wrestlers before and immediately after exercise
SD = Standard Deviation, NO= Nitric oxide, PON-1 = Paraoxonase-1, 8-OHdG = 8-hydroxy-2'-deoxyguanosine

NO levels of the wrestlers increased immediately after a 1.5 hr exercise compared with those in basal status ($p = .002$), no difference was found between the wrestlers basal status and immediately after exercise in PON 1 activity ($p = .408$, Table 2).

Statistically significant correlations were found between the NO level and PON activity in the wrestlers in basal status ($r = .671$, $p = .002$).
DNA glycosylase (hOGG1), which is responsible for the excision of 8-OHdG from the damaged DNA, is increased in human skeletal muscle after a marathon race. This observation suggests that this up-regulation might be the result of regular exercise induced adaptation process (35). It seems to us that the decrease in the level of 8-OHdG in the wrestlers may be an outcome of an adaptation involving up-regulation of 8-OHdG repair enzyme activity due to regular wrestling exercise. However, further studies on DNA damage and DNA repair enzyme in the wrestlers or wrestling exercise are necessary.

Urinary 8-OHdG level has been widely investigated in exercise studies as a biomarker of oxidative stress, and some investigators observed an increase in urinary excretion of 8-OHdG after exercise (1,28,29,32), while others reported no change (15,16). In contrast, the data on serum 8-OHdG level in exercise studies is limited. In serum samples, Tsakiris et al. (42) reported that level of 8-OHdG significantly rose in adolescent male basketball players, after the game, but Bloomer at al (7) found no change in the level of 8-OHdG in cross-trained men after exercise. We found that serum 8-OHdG level in wrestlers did not alter immediately after exercise. These contradictory results may be accounted for by the degree, period and type of the exercise, training status of individuals or adaptation from regular exercise, and the time when samples are taken.

Exercise training is known to induce several adaptations in the cardiovascular system, one of which improves endothelial-dependent vasodilatation. This improvement reflects the increase in bioactivity of NO (14,20,22,24,34,38). Available information on exercise-dependent bioactivity of NO is limited. It is reported that effect of exercise on endothelium-dependent vasodilatation shows the alteration according to intensity of the exercise (14). We observed that NO level in serum samples from wrestlers in basal status showed a significant increase compared with the control group. This result points to a positive effect on endothelium-dependent vasodilatation induced by wrestling exercise. The serum NO levels of wrestlers significantly increased immediately after exercise. NO is a mediator that has a short half-life and may be affected instantly (36). The increase in NO level may be a result of the acute NO production during exercise.

Paraoxonase-1 (PON1) is an enzyme the antioxidant HDL-linked enzyme and it hydrolyses lipid peroxides in oxidized lipoproteins (5,40). The effect of exercise on PON1 activity has been investigated several studies, but the results are contradictory. While exercise increased PON activity in some studies (8,13), no change of PON activity was reported in other studies (23). Tomas et al. (40) found that the effects of regular and acute exercise on PON1 activity levels could be modulated by PON1–192 polymorphism. In our study, the PON1 activity in serum samples from wrestlers in basal status was found significantly elevated compared with the control group. Increased PON activity may be an adaptation response associated with regular exercise. In addition, that the PON1 activity remains unchanged immediately after exercise may show that PON1 activity was not affected immediately after exercise.

The correlation found between NO level and PON1 activity indicate that regular wrestling exercise has a positive effect against oxidative damage, therefore, NO may act as a potential antioxidant agent (4,18). It has been reported that endothelial dysfunction may result from decreased production of NO or inactivation of NO by oxygen-derived free radicals such as O₂ (37). That the NO level was found higher
than those in controls, both before and immediately after exercise in wrestlers, and that NO is positively correlated to PON1, an antioxidant enzyme suggest that there could not exist an endothelial dysfunction, and furthermore that regular wrestling exercise has an antioxidant enzyme related protective effect. Regular wrestling exercise training, perhaps via increased capacity for NO formation and increased PON1 activity retards atherosclerosis, which has significant implications for human health.

Wrestling exercise is an intense exercise that involves both aerobic and anaerobic exercise (27). However, although representing an intense level of physical activity, the wrestling practice protocol is encountered in the lives of many adolescents and is not atypical of intensity found in other high school level individual and/or team sports. We found that regular wrestling exercise in adolescents may improve antioxidant enzyme PON1, NO level, and can be beneficial to inhibit oxidative DNA damage. These demonstrate signed that regular wrestling exercise to reduce oxidative damage may cause positive adaptations for antioxidant defense mechanism. We conclude that regular wrestling exercise for a healthy life is important since it reduces DNA damage while enhancing antioxidant parameters.

Acknowledgments

The authors are grateful to Mustafa Akgul for the linguistic support in preparing the manuscript. Erciyes University Research Foundation has supported this research; Contract grant number BESYO–07–02.

References


