The Effect of Exercise on Serum Homocysteine, Adma, Neopterin and Oxidative Stress Levels in Young Male Athletes

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ABSTRACT

Elevated homocysteine (HCY), asymmetric dimethylarginine (ADMA), Neopterin (NP) and oxidative stress have been established as risk factors for vascular disease and coronary artery disease. The purpose of the study was (a) to assess the HCY, ADMA, NP, malondialdehyde (MDA) and glutathione (GSH) levels and (b) to determine the relationship between these parameters in wrestlers, track and field athletes, and non-athletes. 35 subjects in total, comprising of 15 wrestlers (x̄ = 17.6 ± 2.3 years), 10 track and field athletes (x̄ = 16.6 ± 2.2 years) and 10 non-athletes (x̄ = 17.2 ± 0.8 years) voluntarily participated in the study. The HCY, ADMA, NP, MDA, GSH levels as in subjects' blood was performed through the antecubital vein in the morning while in the pre-prandial state. There was a significant difference in ADMA, MDA, GSH concentrations between in track and field athletes and wrestlers. There was a significant difference in HCY, NP concentrations between wrestlers and non-athletes. There was a significant difference in HCY, MDA concentrations between track and field athletes and non-athletes (p< 0.05). The HCY and NP showed high level correlation in all groups (track and field athletes; r = .952; wrestlers; r = .886; non-athletes; r = .900; p< 0.01). The GSH and NP showed high level negative correlation in non-athletes (r = - .900; p< 0.01). According to the results of HCY, ADMA, NP, MDA, the lowest level was found in athletes and the highest appeared in the non-athletes. In conclusion, we think that regular training may have positive effects on cardiovascular and anti antioxidant level.

KEY WORDS: Asymmetric dimethylarginine, Glutathione, Homocysteine, Malondialdehyde, Neopterin

INTRODUCTION

In all kinds of sports, the athletes are constantly exposed to various forms of stress. The adaptation to this stress occurs in the athlete's body, including subcellular, cellular, tissue and the organs which affect the all the organisms in different levels [1]. Exercise has been known as a significant tool for prevention and treatment of cardiovascular disease (CVD) [2]. Increased oxidative stress in individuals who do irregular exercise with intensive training leads to lipid, protein, and DNA damage [3]. These
individuals could be under a potential risk of cardiovascular disease due to their excessive effort. It is clear that the increase in ADMA level plays a role in many diseases [4]. One of the most important mediators in regulating ADMA endothelial function seems to be the most important endogenous inhibitors of nitric oxide synthase (NOS) [5]. Decreased nitric oxide synthase leads to vasodilatation and atherosclerotic diseases [6-8]. The determination of the ADMA level has been shown to be an early indicator in serum/plasma especially for these diseases [4,9]. Some studies [10,11] suggest that aerobic exercise training may play an important role in reduction of ADMA level. Similarly, HCY has been established as a risk factor for many diseases such as peripheral vascular disease and coronary artery disease [12-15]. Increase in HCY concentration by 5 μmol/L was associated with a significant increase of CVD risk [14,16,17]. Some studies [18-22] reported that the importance of HCY as a potential risk marker for CVD is the unclear debate. In addition this, some studies [23,24] also state that genetics, age, gender, drug use, lifestyle factors such as diet, alcohol, smoking and coffee intake and exercise also influence HCY levels. Some of these studies [13,25] focused on HCY only in terms of effects on acute and chronic exercise. The studies reported that while acute exercise seems to induce an intensity and duration-dependent HCY increase, and in chronic exercise, it is less clear that intensive exercise seems to stimulate an important HCY increase that probably depends on duration and intensity. However, it appears that there is a lack of studies on this topic. NP has been released from monocytes/macrophages that are stimulated by the action of T lymphocytes and IFN gamma in the active cellular immune system [26]. NP measurement has been used to evaluate the progression of diseases such as infections, renal transplant rejection, systemic diseases and some autoimmune diseases [27].

NP has been used as a new method of identifying an acute inflammatory response in exercise [28]. In long duration exercises, it could lead to different changes in acute phase response in the activation of immune system. Physical exercises cause some changes in body homeostasis, increasing NP levels. The possible changes in neopterin are similar in some exercise types (straight cruising and bicycle marathon, mountain walk) [26]. Strasser et al. [29] reported that exercise-induced inflammation and immune activation were important effects on biochemical pathways in aerobic exercise but the intensive exercise is also thought to cause a strong rise in NP levels. Malondialdehyde (MDA), the degradation product of lipid peroxides that are damaged by oxidative stress, can react with DNA or proteins and mutagenize. MDA increases in proportion to the severity of lipid peroxidation, but it is not specific and is also used to determine the lipid damage, caused by oxidative stress [30-32]. GSH has been known to play an important role in the prevention of diseases in the antioxidant defense system and in the well-preservation of health. It is clear that exercise plays an important role in activating the antioxidant defense system [33,34].

In reviewing published literature on sports, it was seen that there is the paucity of publications containing biochemical parameters (HCY, ADMA, NP). In this study, it was hypothesized that in athletes the HCY, ADMA, NP would be lower depending on regular training than non-athletes. The purpose of the study was (a) to assess the HCY, ADMA, NP, MDA, and GSH levels, and (b) to determine the relationship between these parameters in wrestlers, track and field athletes, and non-athletes.
METHODS

Subjects
A total of 35 young male athletes voluntarily participated in the study, wrestlers (n=15; average age 17.6 ± 2.3 years; height 168.5 ±7.5 cm; weight 67.4 ± 14.4 kg), track and field athletes (n=10; average age 16.6 ± 2.2; height 169.1 ± 5.9 cm; weight 56.7 ± 6.4 kg) and non-athletes (n=10; average age 17.2 ± 0.8 years; height 171.8±8.7 cm; weight 66.0 ± 5.5 kg). Track and field athletes and wrestlers were asked not to participate in a daily training program within 24 hours prior to testing. Testing was completed for all track and field athletes and wrestlers in the same conditions. The subjects did not take any medication except for non-steroidal anti-inflammatory drugs when needed. All the athletes and non-athletes were healthy: no abnormal levels of blood pressure, no abnormal liver or renal function, and no history of diabetes were found. All athletes were instructed to abstain from caffeine, alcohol, and drug consumption and to refrain from any strenuous physical activity for 24 h before each evaluation session. The session included a pre-prandial blood sampling in the morning and a complete medical exam. All track and field athletes, wrestlers and non-athletes participants’ parents were notified of the research procedures, requirements, benefits, and risks before giving informed consent oral and written consent for the study was obtained from all subjects, and was conducted in a manner consistent with the institutional ethical requirements for human experimentation in accordance with the Declaration of Helsinki. The study was approved by the local Ethics Committee, and Authorization was given by the ethics committee of Ondokuz Mayıs University, Samsun, Turkey (OMU KAEK 2015/181).

Training Protocol
The wrestlers participated in training programs of 1.5-hour exercise in a day, 6 days per week through the training season. The training season was divided into four training parts. The first period, lasting a duration of two months, was basic preparatory, consisting of aerobic, muscle endurance for anatomical adaptation and technique – tactic exercises with an average of 60-75% HR max for all active tasks. The second period, lasting a duration of four months, was general preparatory, consisting of extensive and intensive interval loading for improved V02max, maximal strength, speed, technique - tactic, coordination and balance exercises with an average of 75-85% HR max for all active tasks. The third period, lasting a duration of one month, was specific preparatory, consisting of aerobic-anaerobic power, maximal power, technique – tactic, coordination, balance, reaction, and wrestling workout exercises with an average of 85-100% HR max for all active tasks. The last period, lasting duration of two months, was competition term, consisting of technic and tactic training, preparation match and official match with an average of 85-100% HR max for all active tasks.

The track and field athletes participated in training programs of 1.5-hour exercise in a day, 6 days per week through the training season. The season was divided into three training parts (basic preparatory, pre-competition preparatory term, competition term). In basic preparatory lasting a duration of 6-8 weeks, including basic aerobic and strength workouts (climbing stairs, horizontal and vertical jumping, running coordination, and skipping rope, moderate long duration running) with an average of 50-60% HR max for all active tasks. In pre-competition preparatory term lasting duration of 8-10 weeks, including extensive and intensive interval running with an average of 75-85% HR max for all active tasks. In competition term lasting duration of 6-10 weeks, including
maximal and submaximal running loading with an average of 85-100% HR max for all active tasks.

**Anthropometric Measures**

The participants were weighed with light clothes and without shoes. The weight was determined within 0.1 kg for each subject using an electronic scale calibrated before each measurement session (Seca 664, Hamburg, Germany). The height was determined using a fixed wall-scale measuring device to the nearest 0.1 cm. The body mass index (BMI) was calculated as weight in kg divided by weight in meters squared. Body fat percent was calculated by using the BC-418 8-contact electrode BIA system (Tanita Corp., Tokyo, Japan).

**Blood samples**

The blood samples were collected at the same time from each track and field athletes, wrestlers and control group. All samples were taken in the morning just after the wake-up and before breakfast. The sera samples were prepared, then stored at -80°C (Daihan, Korea) until laboratory analyses were performed.

**Biomolecules parameters**

**Asymmetric Dimethylarginine (ADMA)’ measurement**

ADMA’ measurements were carried out through HPLC using a method used by Chen et al. [35] and developed by Cakir et al. [36] and Avci et al. [37]. The peaks’ areas defined via fluorescent detector (excitations, 338 nm; emission, 425 nm) were used for quantification. The variation’ intra and inter-assay coefficients were 2.8% and 4.5%, respectively.

**Homocysteine’ measurement**

Determination of homocysteine levels for serum of the patients and healthy individuals included in the study was done performed Immuchrom HPLC (Germany) kit in HPLC system (High Performance Liquid Chromatography). This method is based on oxidation-reduction principles. Chromatograms were recorded via a fluorescence detector. Quantifications were performed with the kit-calibrator.

**Malondialdehyde (MDA)’ measurement**

In MDA’ determination, a derivatization step was used, in which protein-bound MDA was hydrolyzed (60 min at 95°C) and converted into a fluorescent probe. The chromatograms were determined via a fluorescence detector. Quantifications were performed by the kit-calibrator. Concentrations were calculated by integration of the peak heights using the following equation:

\[
\text{conc}_{\text{sample}} = \frac{\text{peak height}_{\text{patient}} \times \text{conc}_{\text{calibrator}}}{\text{peak height}_{\text{calibrator}}}
\]

**Glutathione (GSH)’ measurement**

In GSH were measured, glutathione was converted into a fluorescent probe during the derivatization reaction. Chromatograms were determined via a fluorescence detector. In quantifications were used EDTA-blood calibrator and the internal standard (IS) method. Results were calculated through the following equation:

\[
\text{conc}_{\text{sample}} = \frac{[(\text{peak area}_{\text{patient}} \times \text{conc}_{\text{calibrator}}) / \text{peak area}_{\text{IS patient}}] \times F}{\text{F}= \text{peak area IS of the calibrator} / \text{peak area calibrator}}
\]
**Cellular immunity marker**

**Neopterin’ measurement**

Neopterin’ measurements were accomplishment through HPLC using a method described by Cakır et al. [36] and Avcı et al. [37]. The peaks’ areas determine via fluorescent detector (excitations, 353 nm; emission, 438 nm) were used in quantification. The intra and inter assay coefficients of variation for neopterin were 4.7% and 6.9%.

**Statistical Analysis**

All values were presented as the mean (SD). Continuous variables are described as mean ± SD and median (minimum-maximum). Normality of the continuous variables was assessed by Shapiro-Wilk’s test. For the comparing group variables, it was used to Kruskal-Wallis and as a post hoc Mann-Whitney U-test. The relationship between the blood parameters was determined using the Spearman correlation. Calculations were done with the software package SPSS (version 22.0 for windows; package SPSS Inc., USA). The differences were considered statistically significant at p<0.05.

**RESULTS**

General characteristics of track and field athletes, wrestlers and non-athletes were given in Table 1. The comparision of parameters ADMA, HCY, NP, MDA and GSH of within groups were given Table 2. There was a significant difference in ADMA median (H(2)=10.489; p=0.005). This difference was seen to track and field athletes comparing to others (p<0.05) (Table 2), and the highest level was observed in the non-athletes. The literature studies reported that ADMA is an inhibitory nitric oxide synthase; is associated with atherosclerotic and has been shown in many studies [38-40]. Tanahashi et al. [10] showed that ADMA levels are associated with aerobic fitness. And also, they reported that regular aerobic exercise may decrease ADMA levels. The ADMA results in this study were seen to be consistent with the literature findings. Based on these results, it could be said that aerobic exercises affect ADMA level in track and field athletes positively.

There was a significant difference in Neopterin median (H(2)=7.566; p=0.023). This difference was seen to wrestlers and non-athletes (p=0.010). There was a significant difference in MDA median (H(2)=14.774; p=0.001). This difference was seen to track and field athletes and wrestlers; track and field athletes and non-athletes (respectively: p=0.001; p=0.003). There was a significant difference in GSH median (H(2)=5.950; p=0.05). This difference was seen to track and field athletes and wrestlers (p=0.031). The correlations of some blood parameters were given in Table 3. The HCY and NP showed high level correlation in track and field athletes, wrestlers and non-athletes (r=0.952; r=0.886; r=0.900 respectively) (p<0.01). The GSH and NP showed negative high level correlation in non-athletes (r = -0.900) (p<0.01).

**DISCUSSION**

In the present study, we aim to evaluate cardiovascular risk factors and some oxidative stress parameters in young male athletes such as track and field athletes and wrestlers. According to the results of ADMA, the lowest level was found in track and field athletes comparing to others (p<0.05) (Table 2), and the highest level was observed in the non-athletes. The literature studies reported that ADMA is an inhibitory nitric oxide synthase; is associated with raised cardiovascular diseases’ risk and this has been shown in many studies [38-40]. Tanahashi et al. [10] showed that ADMA levels are associated with aerobic fitness. And also, they reported that regular aerobic exercise may decrease ADMA levels. The ADMA results in this study were seen to be consistent with the literature findings. Based on these results, it could be said that aerobic exercises affect ADMA level in track and field athletes positively. Some studies [41,42] found a positive association between CVD and inflammatory mediators and...
increased HCY is proposed to influence cardiovascular risk by promoting increased oxidative stress. Few studies focus on HCY levels and oxidative stress in individual sports such as wrestling and running, in which exercises follow periods of endurance training and intermittent anaerobic activity at the high vigorous level. In our study, it was revealed that HCY level in non-athletes was seen to be high comparing to the athletes shown in Table 2. It was seen that the lowest value was in track and field athletes, and the highest was in the non-athletes.

Table 1 General characteristics of participant.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Track and field athletes (n=10)</th>
<th>Wrestlers (n=15)</th>
<th>Non-athletes (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>16.6±2.2</td>
<td>17.6±2.3</td>
<td>17.2±0.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.1±5.9</td>
<td>168.5±7.5</td>
<td>171.8±8.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>56.7±6.4</td>
<td>67.4±14.4</td>
<td>66.0±5.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.8±1.7</td>
<td>23.3±3.5</td>
<td>22.4±1.1</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>11.0±3.5</td>
<td>9.8±5.1</td>
<td>10.4±2.5</td>
</tr>
<tr>
<td>TE (yr)</td>
<td>2.2 ± 1.7</td>
<td>4.3 ± 1.4</td>
<td></td>
</tr>
</tbody>
</table>

BMI: Body mass index; TE: Training of experience.

Table 2 The comparision of parameters ADMA, HCY, NP, MDA and GSH of within groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>G</th>
<th>Median</th>
<th>Min.</th>
<th>Max.</th>
<th>p</th>
<th>U</th>
<th>Z</th>
<th>Post hoc p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>1</td>
<td>.51</td>
<td>.41</td>
<td>.80</td>
<td>18.00</td>
<td>-3.162</td>
<td>.002</td>
<td>(1-2)*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>2</td>
<td>.67</td>
<td>.51</td>
<td>1.39</td>
<td>0.005*</td>
<td>23.00</td>
<td>-1.266</td>
<td>(2-3)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>3</td>
<td>1.22</td>
<td>.057</td>
<td>1.49</td>
<td>10.00</td>
<td>-1.837</td>
<td>.066</td>
<td>(3-1)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>1</td>
<td>14.29</td>
<td>5.16</td>
<td>19.16</td>
<td>63.00</td>
<td>-.666</td>
<td>.506</td>
<td>(1-2)</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>2</td>
<td>11.47</td>
<td>7.75</td>
<td>86.18</td>
<td>0.033*</td>
<td>8.00</td>
<td>-2.575</td>
<td>(2-3)*</td>
</tr>
<tr>
<td>Homocysteine (µmol/l)</td>
<td>3</td>
<td>16.83</td>
<td>14.98</td>
<td>52.18</td>
<td>9.00</td>
<td>-1.960</td>
<td>.050</td>
<td>(3-1)*</td>
</tr>
<tr>
<td>1</td>
<td>9.88±2.80</td>
<td>9.15</td>
<td>4.90</td>
<td>14.30</td>
<td>49.500</td>
<td>-1.415</td>
<td>.157</td>
<td>(1-2)</td>
</tr>
<tr>
<td>Neopterin (nmol/L)</td>
<td>2</td>
<td>8.00</td>
<td>.49</td>
<td>17.90</td>
<td>0.023*</td>
<td>8.00</td>
<td>-2.577</td>
<td>(2-3)*</td>
</tr>
<tr>
<td>3</td>
<td>15.76±8.61</td>
<td>10.30</td>
<td>9.80</td>
<td>29.30</td>
<td>11.500</td>
<td>-1.655</td>
<td>.098</td>
<td>(3-1)</td>
</tr>
<tr>
<td>MDA (µmol/l)</td>
<td>1</td>
<td>.96±0.1</td>
<td>.97</td>
<td>.84</td>
<td>1.05</td>
<td>16.500</td>
<td>-3.249</td>
<td>.001(1-2)*</td>
</tr>
<tr>
<td>2</td>
<td>1.08±0.1</td>
<td>1.20</td>
<td>.93</td>
<td>9.70</td>
<td>0.001*</td>
<td>21.000</td>
<td>-1.442</td>
<td>.149 (2-3)</td>
</tr>
<tr>
<td>3</td>
<td>1.47±0.3</td>
<td>1.36</td>
<td>1.05</td>
<td>1.94</td>
<td>.500</td>
<td>-3.009</td>
<td>.003</td>
<td>(3-1)*</td>
</tr>
<tr>
<td>1</td>
<td>200±140</td>
<td>185.05</td>
<td>38.20</td>
<td>510.00</td>
<td>36.000</td>
<td>-2.163</td>
<td>.031</td>
<td>(1-2)*</td>
</tr>
<tr>
<td>GSH (µmol/l)</td>
<td>2</td>
<td>373.00</td>
<td>84.00</td>
<td>482.80</td>
<td>0.05*</td>
<td>18.00</td>
<td>-1.702</td>
<td>.089 (2-3)</td>
</tr>
<tr>
<td>3</td>
<td>315±77</td>
<td>188.60</td>
<td>154.40</td>
<td>349.60</td>
<td>21.000</td>
<td>-.490</td>
<td>.624</td>
<td>(3-1)</td>
</tr>
</tbody>
</table>

*p<0.05; G: Group, 1: Track and field athletes; 2: Wrestlers, 3: Non-athletes; Min: Minimum, Max: Maximum; HCY: Homocysteine; MDA: Malondialdehyde; GSH: Glutathione; ADMA: Asymmetric dimethylarginine.
Table 3. The correlations of some blood parameters.

<table>
<thead>
<tr>
<th>Track and field athletes</th>
<th>Neopterin</th>
<th>MDA</th>
<th>GSH</th>
<th>HCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADMA</td>
<td>-.273</td>
<td>-.304</td>
<td>.370</td>
<td>-.370</td>
</tr>
<tr>
<td>NP</td>
<td>.304</td>
<td>.309</td>
<td>.952&quot;</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td></td>
<td>.043</td>
<td>.426</td>
<td></td>
</tr>
<tr>
<td>GSH</td>
<td></td>
<td></td>
<td>.139</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wrestlers</th>
<th>Neopterin</th>
<th>MDA</th>
<th>GSH</th>
<th>HCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADMA</td>
<td>-.377</td>
<td>.063</td>
<td>-.182</td>
<td>-.218</td>
</tr>
<tr>
<td>NP</td>
<td>-.254</td>
<td>-.208</td>
<td>.886&quot;</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td></td>
<td>-.143</td>
<td>-.306</td>
<td></td>
</tr>
<tr>
<td>GSH</td>
<td></td>
<td></td>
<td>-.189</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-athletes</th>
<th>Neopterin</th>
<th>MDA</th>
<th>GSH</th>
<th>HCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADMA</td>
<td>.300</td>
<td>-.821</td>
<td>-.600</td>
<td>.100</td>
</tr>
<tr>
<td>NP</td>
<td>-.359</td>
<td>-.900’</td>
<td>.900’</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td></td>
<td>.616</td>
<td>-.359</td>
<td></td>
</tr>
<tr>
<td>GSH</td>
<td></td>
<td></td>
<td>-.700</td>
<td></td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level

It was seen that the lowest value was in track and field athletes, and the highest was in the non-athletes. According to Unt et al. [43], physically active ex-athletes showed significantly lower total HCY levels in comparison with the sedentary counterparts. Rousseau et al. [44] reported that athletes who performed aerobic exercise had lower levels of homocysteine. These findings are consistent with our results. However, Herrmann et al. [13] reported that endurance exercise induced a significant HCY increase that possibly depends on intensity and duration but, little is known about the effect of training intensity. They state that the impact of this and possible routine applications are unclear and need further investigation. Publications concerning sports and HCY can be divided as those focused on acute, and chronic exercise. While acute exercise seems to induce an intensity and duration-dependent HCY increase, the effect of chronic exercise is less clear. König et al. [45] investigated the influence of extensive endurance training and acute intense exercise on plasma HCY concentrations in 42 well-trained male triathletes. They reported that although intense exercise acutely increased the HCY levels, chronic endurance exercise was not associated with higher HCY concentrations. According to some studies [16,17,46], there are inconsistent data available concerning the association between HCY and exercise. In another study, Real et al. [16] reported that regular nonintensive exercise or training could decrease plasma HCY concentrations, but intensive prolonged exercise has the contrary effect. Results of our study revealed that the HCY level was higher in the non-athletes than the track and field athletes. According to Hüblner-Woźniak et al. [14], HCY in the
sedentary group was 11.12 ± 2.88, while wrestlers group showed 6.14 ± 1.52 (µmol/L) (p<0.001). These findings are consistent with our results (Table 2). Strength-speed training practiced by elite wrestlers, associated with significantly lower values of HCY in them compared to the untrained subjects, may reduce the risk of cardiovascular diseases at later age, as in case of endurance training [14]. In the general population, plasma HCY concentrations increase throughout life. Several lifestyle factors (smoking, alcohol intake, and coffee intake etc.), raise blood pressure, and some medicine intake may increase the total homocysteine level of the plasma [23,24]. Some studies [25,47] reported that there are not any consistent findings relating to HCY, physical activity, and cardiorespiratory fitness. Our study and literature findings indicate that, as dependent on the fitness level of the participants, HCY level could be influenced by the duration, intensity, and type of exercise.

According to the results of NP, the lowest level was found in wrestlers compared to the others (p<0.05) (Table 2), and the highest was shown in the non-athletes. Increased NP levels are detected in diseases with an inflammatory condition or a highly intense physical exercise [48]. In recent years, the number of researches on plasma and urine neopterin concentrations, especially in the athletes, has been increasing after high-efficiency exercise [28,49]. Increased NP levels are associated with elevated oxidative stress and inflammatory diseases that result in muscle damage [28]. The study findings appeared that the lower MDA and NP in the athletes comparing with non-athletes could be improved based on a chronic adaptation of training.

According to the results of MDA, the lowest level was found in track and field athletes comparing with the others (p<0.05) (Table 2), and appeared to be the highest in the non-athletes. In literature studies, Hadžović-Džuvo et al. [50] reported that a statistically significant difference was shown in the level of MDA of basketball players, who had considerably high levels of MDA in comparison to footballers. Based on this finding, they stated that type of sports has no impact on the levels of oxidative stress markers. Stankovic & Radovanović [51] suggest that increased production of RONS, as well as oxidative stress occurs, in top-quality athletes under maximal physical exertion is independent of an energetic requirement of sports type such as aerobic, anaerobic. Shi et al. [34] reported that the study investigating the differences in oxidative stress caused by aerobic and anaerobic exercise due to different mechanisms suggests that similar workloads of anaerobic and aerobic exercise induce ROS differently: aerobic exercise seems to initially generate more ROS, whereas anaerobic exercise may induce prolonged ROS generation. Although more oxygen was consumed during aerobic exercise, generated ROS did not induce significant oxidative damage, so they concluded that oxygen consumption per individual may not be the major cause of exercise-induced oxidative damage. Demirkan [52] stated that wrestlers have higher concentrations of MDA compared to sedentary subjects. Different impact on oxidative stress during acute and prolonged training was also shown by some studies. Pesic et al. [53] evaluated oxidative status in elite karate fighters during a training session and their results showed that in the long run a programmed physical exercise does not lead to oxidative stress, but an excessive physical load may cause its occurrence. It was seen that these results [34,50,51] are consistent with our study finding that MDA level is lower in track and field athletes than the wrestlers. However, there is a conflict between the findings of our study and that of Demirkan [52]. Depending on this finding, we suggest that the sport of wrestling requires very complex training forms, including maximal strength,
aerobic endurance, and anaerobic capabilities, but the track and field athletes training regimen requires more aerobic capabilities. As a result of this, our study may support that dominant aerobic training leads to a decrease in MDA levels as compared to other training forms, including maximal strength and anaerobic capabilities.

As per another finding of this study, GSH was found as the highest of the wrestlers comparing the others (p<0.05)(Table 2). Similar results have been observed by Hamurcu et al. [54] who found that regular wrestling exercise in adolescents improves antioxidant enzyme which can be beneficial for inhibiting oxidative DNA damage. This result reflects an adaptation of the antioxidant system under the training effects of exercise. But Hadžović-Džuvo et al. [50] reported that mean total antioxidative capacity concentration did not differ among soccer players, wrestlers, and basketball players. These findings were seen to be consistent with our study and Hamurcu et al. [54]. Kantorowicz et al. [55] reported that reduced GSH is a naturally occurring endogenous antioxidant. According to Unt et al [43], physically active ex-athletes showed a significantly lower GSH in comparison with the sedentary counterparts. Intense exercise can cause intensification of ROS production, and thus lead to oxidative stress. However, it should be noted that ROS production depends on not only exercise intensity but also many other factors, including the type and duration of the exercise, gender, and age [55]. Mrakic-Sposta et al. [49] stated that the antioxidant capacity was not considered related to the exercise type. In a similar another study, Trivic et al. [56] reported that there was no evidence that endurance exercise enhanced the levels of any antioxidant enzyme activity. Based on these studies [42,43,49,50], the results of these studies showed that there were many contradicting findings. As for the causes of these

The correlation results in this study showed that high-level correlation was seen between HCY and NP among all groups (p<0.05)(Table 3). In addition to this, the GSH and NP showed a negative high-level correlation in non-athletes (p<0.05) (Table 3). When the literature studies were examined, not any study revealing the relationship between HCY and NP, was found. But, Schobersberger et al. [26] reported that physical exercises that caused some the changes in body homeostasis, increase the NP levels. When the other parameters were highlighted, Unt et al. [43] reported that HCY metabolism was closely related to the GSH, which was important in the protection of cells against free radicals. Powers et al. [42] reported that an analysis of the correlation between HCY and MDA, they observed a strong association between HCY and MDA in young men. These conflicting results may be explained by the type of sport, duration of a training period, training frequency (hr/week) and the specificity of training loads applied to athletes and the physiological characteristics of subjects studied.

**Conclusion**

According to our hypothesis, in this study it was appeared that in athletes the HCY, ADMA, NP could be lower depend on regular training than non-athletes. Exercise has variable effects on the immune system. In conclusion, we may suggest that regular exercise training could have positive effects on cardiovascular and anti antioxidant level. In addition to these, our study was limited by its sample size which should be taken into consideration when drawing
conclusions. However, we did not monitor diets, so the results should be analyzed with caution. HCY level is being affected by several factors and depends on the nutritional status of the athlete, the exercise load and the metabolic factors etc. Further studies are needed to understand the effect of age, gender, branch, duration of training, intensity etc. cardiovascular risk factors.

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Conflicts of interest

The authors declare no conflict of interest for this study.
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