



## Effects of a Volleyball Match on Serum Nitric Oxide Level and Oxidant/ Antioxidant Status

### *Bir Voleybol Maçının Serum Nitrik Oksit Düzeyi ve Oksidan/Antioksidan Durum Üzerine Etkileri*

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#### ABSTRACT

**Objective:** Nitric oxide (NO) is a gas with vasodilator, antioxidant and metabolic regulatory effects. However, NO may be converted into an oxidant substance, under oxidative stress conditions as severe exercise, reducing the bioavailability of NO. Therefore, NO may take part in training adaptations. The main aim of this study was to investigate the effects of a volleyball match on serum NO level and oxidant/antioxidant status, as well as some physiological stress responses.

**Materials and Methods:** Healthy female competitive volleyball players (n=12), and a control group (n=12) that gave up regular volleyball training at least three years prior to the study, aged 16-22 years old, carried out spike-block jump and agility T-tests, and lactate elimination speed was determined following a Yoyo intermittent recovery test. Heart rate (HR), fingertip blood lactate levels, rate of perceived exertion (RPE) were measured during volleyball matches. Serum NO level was determined spectrophotometrically with the "Griess Reaction" method. Serum total oxidant status and total antioxidant status (TAS), erythrocyte glutathione peroxidase activity, and physiological stress markers such as serum creatine kinase (CK), lactate dehydrogenase (LDH), alanine- (ALT) and aspartate amino transferase (AST) activities by enzymatic-colorimetric methods, using venous blood samples taken before and following the matches.

**Results:** The players' HR, lactate and RPE levels during a match were significantly lower than those of the controls. Post-match serum TAS, CK, LDH and AST levels were significantly higher than baseline values for both player (p≤0.05) and control (p≤0.01) groups. However, post-match serum NO levels were higher than pre-match levels only in the player group (p≤0.05).

**Conclusion:** Increased NO and lowered physiological stress levels following a match may result from a higher vasodilator and recovery capacity based on training adaptation in the players, as well as the low intensity of the matches. The observation that serum NO levels did not display relationships with performance parameters may result from the anaerobic nature of volleyball.

**Key Words:** Nitric oxide, oxidative stress, physiological stress, lactate elimination

#### ÖZ

**Amaç:** Nitrik oksit (NO) vazodilatör, antioksidan ve metabolik regülatör özelliklere sahip bir gazdır. Ancak, NO şiddetli egzersizin oksidatif stres koşullarında oksidan bir maddeye

dönüşerek biyoyarlılığı düşebilir. Bu özellikleriyle NO, antrenman adaptasyonlarında önemli rol oynayabilir. Bu çalışmanın temel amacı, bir voleybol maçının serum NO düzeyleri ve oksidan/antioksidan durum üzerine etkilerinin incelenmesidir. Bunun yanısıra voleybol maçına ait bazı fizyolojik yanıtlar da incelenecektir.

**Gereç ve Yöntemler:** Çalışmaya sağlıklı, kadın, 16-22 yaş, antrene voleybolcular (n=12) ile en az üç yıldır düzenli voleybol antrenmanı yapmayan kontrol grubu (n=12) katıldı. Katılımcılara smaç-blok sıçrama ve T-testlerinin yanı sıra Yoyo aralıklı toparlanma testi sonrasında laktat eliminasyon hızı belirlenmesi uygulandı. Grupların kendi içlerinde yaptığı voleybol maçları sırasında kalp atım hızı (KAH), parmak ucundan laktat düzeyi ve egzersizde algılanan zorluk düzeyi (EAZD) belirlendi. Maç öncesi ve sonrası venöz kan örneklerinde serum NO düzeyi, nitratın "Griess Reaksiyonu" yöntemi ile spektrofotometrik olarak saptandı. Serum total oksidan statüsü ve total antioksidan statüsü (TAS) ve eritrosit glutatyon peroksidaz düzeyleri ve fizyolojik stres belirteçleri olarak; serum kreatin kinaz (CK), laktat dehidrogenaz (LDH), alanin amino- (ALT) ve aspartat aminotransferaz (AST) enzim aktiviteleri enzimatik-kolorimetrik yöntemlerle oto analizör aracılığı ile belirlendi.

**Bulgular:** Sporcuların maç sırasında ölçülen KAH, laktat ve EAZD değerleri kontrol grubundan anlamlı olarak düşüktü. Voleybol maçları sonrasında; serum TAS, CK, LDH, AST düzeyleri hem sporcu ( $p \leq 0.05$ ) hem de kontrol grubunda ( $p \leq 0.01$ ) bazal değerlere göre anlamlı düzeyde arttı. NO düzeyleri sadece sporcu grubunda maç öncesine göre anlamlı olarak yüksekti ( $p \leq 0.05$ ).

**Sonuç:** Sporcu grupta maç sonrası artan serum NO ve azalan fizyolojik stres düzeylerinin gözlenmesi, antrenman adaptasyonları ile kazanılan yüksek vazodilatör ve antioksidan kapasiteye ve maçın yoğunluğunun düşük olmasına bağlanabilir. Serum NO düzeyi ile performans parametreleri arasında anlamlı korrelasyonların bulunmaması voleybolun anaerobik doğası ile ilişkili olabilir.

**Anahtar Sözcükler:** Nitrik oksit, oksidatif stres, fizyolojik stres, laktat eliminasyonu

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## INTRODUCTION

Volleyball is an intermittent sport requiring players to compete in frequent short bouts of high-intensity sprints, jumps (blocking and spiking), and court movements during a total of ~60-90 min duration. It requires players to have well-developed anaerobic alactic and glycolytic energy systems, as well as reasonably well-developed oxidative capabilities (1,2). However, the proportion of lactic acid accumulation increase during the repetitive explosive movement pattern is not clear. It is reported that aerobic fitness enhances recovery from high intensity intermittent exercise through increased aerobic response, improved lactate (La) removal and enhanced creatine phosphate (CP) regeneration (3).

Nitric oxide (NO) is associated with the physical fitness status, and it is synthesized from L-arginine through the nitric oxide synthase enzyme (NOS) and is a gas that has vasodilator, antioxidant and metabolic regulatory features (4). Metabolic features of NO during exercise can be summarized as the regulation of blood flow, uptake of glucose into the cell, muscle contraction function and

restriction of glycolysis, mitochondrial respiration and CP break-down. NO is stated to have both oxidant and antioxidant features depending on physiological conditions (4).

Exercise-induced oxidative stress (OS) might itself be beneficial by inducing arterial antioxidant enzymes and improvement in endothelial function has been ascribed to an enhancement in the expression of enzymes associated with antioxidant defense, such as catalase and endothelial NOS (eNOS) as response to moderate-intensity aerobic exercise (5,6), while high intensity exercise increases OS levels by reactive oxygen species generated and affects endothelial function negatively, decreasing the bio-availability of NO (6). All isoforms of NO can be regulated by transcription through hypoxia and muscle contractions. Neuronal NOS (nNOS) expression increases with contusion, severe injury, and muscular activity (4,7).

Possible traumas resulting from repetitive jumps and strength requirement movements may cause NO production during a volleyball match. The increase in NO after volleyball trainings may contribute to volleyball performance, increasing

La elimination and insulin-independent glucose intake because of its role in aerobic fitness or it may restrict volleyball performance as it inhibits phosphocreatine breakdown (4). Therefore, changes in blood NO levels during a volleyball match may affect OS and physiological stress levels either positively or negatively.

Thus, it was hypothesized that a volleyball match increases serum NO levels, and this increase is related to oxidative and physiological stress levels, as well as blood La concentration and La elimination speed (LES), and but not anaerobic power performance indices.

To test the hypothesis, two separate volleyball matches in either young female volleyball players or sedentary controls were compared to determine the effects a volleyball match on serum NO levels and mentioned OS markers, total oxidant status (TOS) and total antioxidant status (TAS), erythrocyte glutathione peroxidase (GPx) activity, albumin and uric acid levels as well as serum creatine kinase (CK), lactate dehydrogenase (LDH), alanin aminotransferase (ALT) and aspartate amino transferase (AST) activities, La concentration, LES and some volleyball performance indices.

## MATERIAL and METHODS

### Subjects

A group of twelve healthy female competitive volleyball players and a control group (n=12), within the age range of 16-22 years volunteered to participate in the study. At the time of the study, volleyball players from two local volleyball clubs, had been training for 6.83(1.69) years with a mean of 10 h/wk in the active season. The control group consisted of volleyball training-experienced subjects that gave up regular volleyball training at least three years prior to the study. None of the participants suffered from any injury or were under any medication. All tests were performed in standard conditions, within a period of seven days in spring during the regular volleyball season, to minimize the effects of training or periodization. In addition, the testing

time of the day was replicated to minimize any effect of circadian variance for each participant. Blood samples were taken in the luteal phase of the menstrual cycle. The study protocol was approved by the Local Medical Research Ethics Committee (11-3.1/10-02.06.2011) and written informed consent was obtained from the parent (for participants age <18 years).

### Experimental Design

Pre- and post-test study design was used for this standardized controlled court experiment in order to investigate the effects of a volleyball match on physiological and OS markers. In the same day, spike-block jump and agility T-test, Yoyo intermittent recovery, LES and heart rate recovery speed tests were conducted to evaluate participants' athletic status by determination of jump heights (cm), test durations (min), running distances (m), fingertip blood La responses (mM) and heart rates (HR) (beats/min). Following a period of three days, three-set training matches were separately performed by active volleyball players, and the control group, one day apart. Venous blood samples taken pre and post-match were used to carry out biochemical analyses.

**Spike-block jump and agility T-test:** Anaerobic capabilities were evaluated by spike and block jump performances (8) and T-test (9) by using standard methods. Each spike and block jump trial was performed three times with an interval of 2 min, and the agility T-test was repeated twice. The highest scores were recorded.

**Yoyo intermittent recovery test (IR1):** IR1 test focuses on the maximal activation of the aerobic system. The test consists of 20-m shuttle runs performed at increasing velocities with 10-s of active recovery (consisting of 2x2.5-m jogging) between runs until exhaustion and 20-min passive recovery following the runs. Test results were recorded as covered distances (m). LES and HR recovery speed (HRRS) were calculated by using recovery La and HR responses as follows:

Capillary blood samples (150 µl) were taken from fingertips at rest, 3<sup>rd</sup> and 20<sup>th</sup> minutes of post-IR1 test. Total blood La (plasma + erythrocytes)

concentrations were analyzed using electro-enzymatic membrane method with a lactate analyzer (YSI 1500 Sport, Yellow Springs Inc., Ohio, USA). LES (mM/min) was calculated by dividing the delta difference in La responses between 3 and 20 min post-test, by the elapsed time of 17 min. HRs were recorded by a telemetric gauge system (Activio AB, Stockholm, Sweden) providing wireless data transmission. HRRS (beats/min) was calculated by dividing the delta difference in HR responses between immediately after and 3<sup>rd</sup> min after the test by the elapsed time of 3 min.

**Volleyball match:** Three-set training matches were played separately by each group, in the presence of a referee. Each player played the whole game without any substitution. Rate of perceived exertion (RPE) according to a Borg's category-ratio scale (10), HR and La were evaluated at 5<sup>th</sup> min before, during technical time outs (8<sup>th</sup> and 16<sup>th</sup> points) and immediately after the matches.

### Biochemical Analyses

Totally 15 ml blood samples were collected into evacuated plain (10 ml) for serum, heparinized tubes (2.5 ml) for haemolysate and K<sub>3</sub>EDTA-containing tubes (2.5 ml) for haemogram 30 min before and 10 min after the match. Vacutainer tubes (10 ml) were held at room temperature for 30 min and then centrifuged for 15 min at 1500 g. Serum was removed and kept at -20°C until analysis. The analyses were performed within five days. Participants' fasting blood glucose, blood urea nitrogen, uric acid, creatinine, total protein, albumin, globulin levels, ALT and AST enzyme activities; serum NO levels, CK and LDH activities, serum total antioxidant (TAS) and oxidant status (TOS), and erythrocyte GPx activity were analyzed through an auto-analyzer (Abbott Architect C8000, USA). Parameters were assessed to reveal the effects of the volleyball match on those markers. All samples were analyzed in the same day to minimize the influence of analytical variation. Changes in blood volume (PV, %) were calculated by Dill and Costill's method (11) and corrected. Haemogram analysis from an EDTA tube was performed at a special laboratory by an automatic hematological

analyzer (BC-3200 Mindray, China) within 3-4 hours in the same day.

**Analysis of serum NO levels:** NO analyses were performed spectrophotometrically (Shimadzu UV 1700, Japan) using a commercial kit (Oxford, Biomedical Research, USA) by the Griess reagent. Serum NO levels (NO<sub>2</sub> + NO<sub>3</sub>) were calculated as μM. Within-run coefficient of variation (CV) was <9.0% for NO.

**Measurement of TAS and TOS levels:** TAS reveals the total antioxidant capacity of the body against powerful free radicals, whereas TOS gives total oxidant amount in the body. They were determined with a commercial kit (Rel Assay Diagnostics, Turkey) spectrophotometrically by an auto-analyzer (Abbott Architect C8000, USA) using a colorimetric method developed by Erel (12,13) in serum samples. Results were given as (mmol trolox Eqv/l). The within-run CV was 1.4% for TAS. For TOS, results are given as the equivalent of H<sub>2</sub>O<sub>2</sub> (μmol H<sub>2</sub>O<sub>2</sub> Eqv/l). Within-run CV was 3.6% for TOS. The ratio of serum TAS to TOS is defined as the oxidative stress index (OSI).

**Analysis of GPx activity:** Measurement of erythrocyte GPx activity from the haemolysate was kinetically performed by the method of Paglia and Valentine (14) using a commercial kit (Randox Laboratories, UK) by auto-analyzer (Abbott Architect C8000, USA). To prepare the haemolysate, heparinized blood samples were firstly washed three times with physiological saline and stored in deep freezer at -20°C until the erythrocyte samples were analyzed. Before analysis, the washed erythrocyte was allowed to dissolve at room temperature, and then it was haemolysed using a Drapkin solution. Results were given as (U/g Hb). The within-run CV was 4.5% for GPx.

### Statistical Analysis

Data were analyzed using SPSS v15.0 (SPSS Inc., Chicago, USA), following normality (Kolmogorov-Smirnov) and homogeneity (Levene) testing. Wilcoxon's signed rank test was used to compare pre- and post-test values. Comparisons between each dependent variable were made by the Mann-Whitney U test. Correlation analyses were performed by Spearman's rho test. Significance

was established at the level of  $p \leq 0.05$ . Data were given as mean (SD).

## RESULTS

Descriptive analyses are given in Table 1. Accordingly, active players had significantly higher

spike, block jump height (cm) and agility T-test scores (s) compared with the control group. Post-IR1 La and post-IR1 HR responses at the 3<sup>rd</sup> min were significantly lower in volleyball players than the controls (Table 2).

**Table 1.** The characteristics of the participants

Parameters	Players (n=12)	Controls (n=12)
Age (year)	17.0(1.04)***	20.3(1.49)
Height (cm)	171(5.99)	170(3.78)
Weight (kg)	62.1(5.96)	60.3(6.14)
BMI (kg/m <sup>2</sup> )	21.2(1.94)	21.0(2.31)
Body fat ratio (%)	21.2(4.66)	21.8(5.02)

\*\*\*:  $p \leq .001$

**Table 2.** Comparison of some physiologic parameters for players and controls

Parameters	Players (n=12)	Controls (n=12)
Spike jump height (cm)	42.7 (0.11)*	36.6(0.10)
Block jump height (cm)	32.6 (0.13)*	25.5(0.11)
Agility T-test (sec)	10.7 (0.57)*	11.5(0.50)
Post-IR1 La 3 <sup>th</sup> min (mM)	12.5 (2.41)*	14.9(2.63)
Post-IR1 La 20 <sup>th</sup> min (mM)	8.87 (3.14)	11.8(3.29)
LES (mM/min)	0.21 (0.10)	0.18(0.09)
IR1 max HR (beats/min)	177 (17.2)	187(12.3)
Post-IR1 HR 3 <sup>th</sup> min (beats/min)	122 (11.1)*	136(11.6)
HRRS (beats/min)	18.4 (5.10)	17.2(4.65)
IR1 total distance (m)	598.3 (226.4)***	457(104)

IR1: Yoyo intermittent recovery test, LES: Lactate elimination speed, HR: heart rate, HRRS: heart rate recovery speed; \*:  $p \leq 0.05$ , \*\*\*:  $p \leq 0.001$

Average fingertip blood La, HR and RPE measured during the match were significantly higher in the control group ( $3.7 \pm 1.0$  mM,  $132.9 \pm 16.5$  beats/min, 4.25) when compared with the players ( $2.8 \pm 0.4$  mM,  $105.3 \pm 12.8$  beats/min, 3.40), ( $p \leq 0.05$ ). La, HR and RPE belonging to measuring points are given in Figure 1.

According to main results, there were no significant differences between basal serum

parameters obtained from both players and controls, except CK ( $p=0.02$ ) and albumin ( $p=0.003$ ) levels. Post-test CK ( $p=0.015$ ), LDH ( $p=0.004$ ), TAS ( $p=0.036$ ), NO ( $p=0.012$ ), albumin ( $p=0.028$ ) and AST ( $p=0.008$ ) levels were significantly higher than the basal levels for the player group, while CK ( $p=0.008$ ), LDH ( $p=0.006$ ), TAS ( $p=0.002$ ) and AST ( $p=0.028$ ) levels were significantly higher than the basal levels in the control group (Table 3).

When haematological parameters of both groups' pre-and post-match values were analyzed, there were significant differences in white blood cell count (WBC) in both volleyball players (p=0.005) and the control group (p=0.025), and in the haematocrit (Hct) level only in the controls (p=0.003).

There were no significant correlations between NO and any other parameters obtained from both player and control groups before and after the match.

**Table 3.** Comparison of physiological, oxidative stress and antioxidant markers of player and control groups measured pre- (1) and post-match (2)

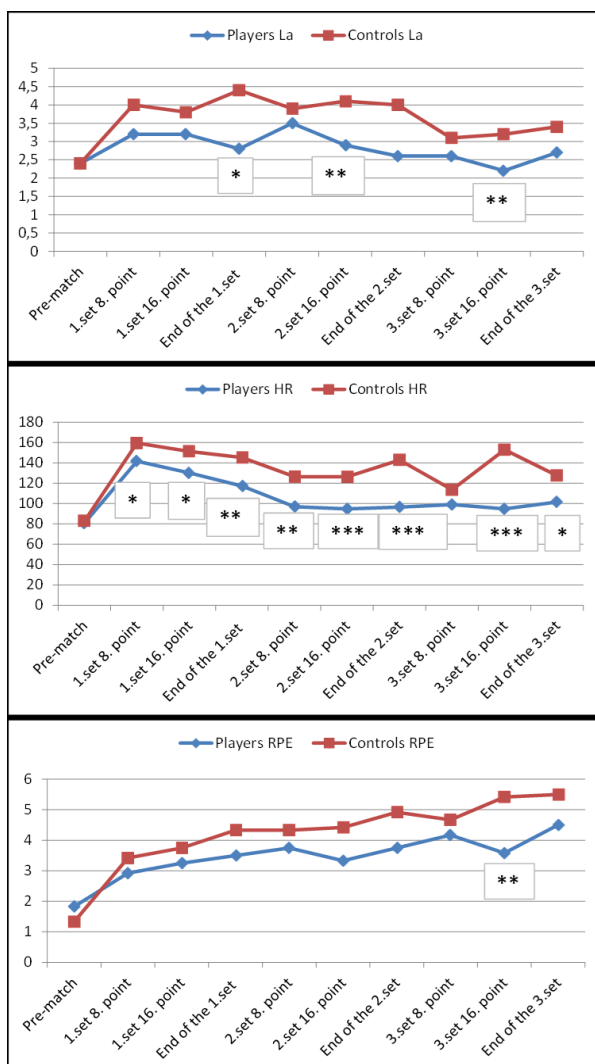
Parameters	Players (n=12)	Controls (n=12)
<b>NO 1</b> (µM)	102 (41.0)	94.8 (42.1)
<b>NO 2</b>	112 (43.5) <b>x*</b>	101 (42.3)
<b>CK 1</b> (U/l)	120 (70.8) <b>a*</b>	72.0 (21.0)
<b>CK 2</b>	156 (101) <b>x*</b>	97.5 (28.5) <b>y**</b>
<b>LDH 1</b> (U/l)	160 (18.8)	153 (16.7)
<b>LDH 2</b>	183 (26.5) <b>x**</b>	167 (22.8) <b>y**</b>
<b>GPx 1</b> (U/g Hb)	74.5 (29.8)	71.1 (16.1)
<b>GPx 2</b>	77.7 (26.6)	72.4 (17.1)
<b>TAS1</b> (mmol Trolox Eqv/l)	1.58 (0.13)	1.51 (0.08)
<b>TAS 2</b>	1.61 (0.16) <b>x*</b>	1.56 (0.08) <b>y**</b>
<b>TOS1</b> (µmol H <sub>2</sub> O <sub>2</sub> Eqv/l)	2.04 (0.46)	2.31 (0.66)
<b>TOS 2</b>	2.35 (0.81)	2.49 (0.80)
<b>OSI 1</b>	1.32 (0.35)	1.53 (0.44)
<b>OSI 2</b>	1.47 (0.51)	1.60 (0.50)
<b>Uric acid 1</b> (mg/dl)	4.03 (0.75)	3.83 (0.65)
<b>Uric acid 2</b>	4.06 (0.84)	4.04 (0.62)
<b>Albumin 1</b> (mg/dl)	4.57(0.18)	4.90 (0.30) <b>a**</b>
<b>Albumin 2</b>	4.81 (0.24) <b>x*</b>	4.89 (0.49)
<b>AST 1</b> (U/l)	16.7 (2.38) <b>a*</b>	13.3 (3.53)
<b>AST 2</b>	18.5 (3.53) <b>b* x**</b>	15.3 (3.03) <b>y*</b>
<b>ALT 1</b> (U/l)	9.83 (2.82)	8.67 (3.73)
<b>ALT 2</b>	9.97 (3.36)	9.03 (3.37)

(a) for pre-match (b) for post-match, comparing by Mann-Whitney U test; (x) for player group, (y) for control group, comparison of pre-post match differences (Wilcoxon test). NO: nitric oxide, CK: creatine kinase, LDH: lactate dehydrogenase, GPx: glutathione peroxidase, TAS: total antioxidant status, TOS: total oxidant status, OSI: oxidative stress index, AST: aspartate amino transferase, ALT: alanine amino transferase; \*: p≤ 0.05, \*\*: p≤ 0.01, \*\*\*: p≤ 0.001

**Table 4.** Comparison of haematological parameters of player and control groups measured pre-(1) and post-match (2).

Parameters	Players (n=12)	Controls (n=12)
Hct 1 (%)	35.8 (2.40)	38.6 (4.44)
Hct 2 (%)	35.5 (2.85)	37.7 (4.22) <b>y*</b>
Hb 1 (g/dl)	12.0 (0.88)	12.9 (1.70)
Hb 2 (g/dl)	11.9 (1.15)	12.7 (1.78)
WBC 1 (10 <sup>3</sup> /mm <sup>3</sup> )	6.38 (1.80)	7.93 (2.21)
WBC 2 (10 <sup>3</sup> /mm <sup>3</sup> )	7.31 (1.89) <b>x**</b>	9.05 (2.45) <b>y*</b>
PV (%)	-0.46 (2.1)	-1.3 (2.4)

(x) for player group, (y) for control group, comparison of pre-post match differences (Wilcoxon test). \*: p ≤ 0.05, \*\*: p ≤ 0.01, Hct: haematocrit, Hb: haemoglobin, WBC: white blood cell, PV: plasma volume difference



**Figure 1.** Heart rate (beats/min), fingertip blood lactate (mM) and rate of perceived exertion (1-10) levels measured during the matches for volleyball player and control groups. Marked values show statistical significance (p ≤ 0.05). It was proved that matches created a moderate physiological stress (close to the lactate threshold) according to the HR, La, and RPE levels both for the players (105.3 ± 12.8 beats/min; 2.8 ± 0.4 mM; 3.40) and the controls (132.9 ± 16.5 beats/min; 3.7 ± 1.0 mM; 4.25).

**DISCUSSION**

The main findings of the present study were that volleyball players displayed increased NO levels following a match, and significant relationships among NO levels and other factors were not found in any group, including LES and other physiological and OS parameters as well as volleyball performance indices before and following the game. In this study, WBC, CK, LDH and AST parameters, which were used as the indicators of muscle damage (15), increased significantly after matches in both groups. These findings show that although both of the matches are of moderate intensity, micro traumas occur in volleyball due to many explosive movements in repeated jumps and digs. These results were similar with the study of Souglis et al. (16), which reported that a volleyball match showed the smallest increase in inflammation and muscle damage markers compared with the other team sport matches as basketball, soccer,

and handball. The appearance of lower muscle damage levels may be a result of the volleyball training adaptations (17).

nNOS levels are related to "fast twitch" glycolytic, whereas eNOS levels are associated with oxidative muscles (4). Considering that fast twitch fibres are used more in volleyball and that specific repeated movements in volleyball can cause NO increase, nNOS mediated NO increase is expected dominantly in both player and the control groups. However, only NO levels of the players increased significantly following the match. It ensues that specific energy and movement types in volleyball and physiological stress occurring during a match are not sufficient for NO increase for controls. Therefore, along with the factors specified, an endothelium and antioxidant capacity obtained by regular volleyball training, and its activation during exercise may play a role in the NO increase occurs in the players, in contrast to the controls.

The intensity and duration of the exercise is an important factor for the upregulation of endogenous antioxidants (18). Adaptations developed by the body exposed to repeated stresses lead to improvements in physiological defense, performance and health, according to the "Hormesis Theory" (19). It has been reported that long-term high intensity endurance exercises are more effective in this regulation and 12-week endurance training reduces the free radical production (20). When mixed exercises are considered, it has been claimed that significant improvement occurs in the antioxidant defense system (21); in football (22,23), rugby (24,25), and handball players (26). It has been observed that basal OS levels are low and antioxidant levels are high in comparison with the control group. In a study carried out with female athletes, it was found that OS parameter levels adequately discriminated 68.5% of athletes with various training experience (27). These findings display that regular volleyball training may lead to a greater antioxidant capacity, compared with the sedentary control groups.

Moreover, the duration of playing volleyball must be sufficiently long to trigger sequential adaptive response of the antioxidant defense system. In

this study, only blood TAS, albumin ( $p<0.05$ ) and GPx ( $p>0.05$ ) levels increased compared with baseline values in both groups after the matches. Although there was no significant increase in OSI in both groups, the increase in TAS, albumin and GPx reveal that OS occurring in volleyball matches is balanced by an antioxidant response.

Additionally, the increase in blood albumin levels with antioxidant potential, which was observed only in players after a match, displays that non-enzymatic antioxidants are significantly mobilized during a match in the player group. It was reported that elite athletes had a potent stimulus of oxidative stress that led to the large recruitment of antioxidant defense (28). Hence, this difference may have played a role in the increase in blood NO levels of the volleyball players.

The fact that there was no remarkable increase in TOS may indicate that the intensity of a training match, played by only six players in each team, is not at a sufficient level to provide excessive free radical production. Moreover, in addition to short-term high intensity movements occurring during volleyball matches, the high duration of intervals between rallies, time-outs and time spent for players' substitutions may inhibit OS production.

Exercise training in healthy individuals promotes endothelial function including aerobic, resistance and combined exercise modalities (29). It has been found that especially aerobic exercise increased baseline NO levels, and there were positive relationships between physical fitness and these levels (26,30-33). This correlation may indicate that athletes have a greater endothelial capacity than sedentary groups. In the study of Ozkol et al. (34), although statistically not significant, basal NO levels of trained male swimmers were higher compared with those of volleyball players and the control group. The baseline NO levels of volleyball players, on the other hand, were not different from those of the control group (34), similar to the findings of this



study. In other studies, baseline NO levels of recreational football players (32) and professional male football players (35) were higher than that found in groups either participating in jogging or living a sedentary life. These differences were attributed to the greater anaerobic quality of football (e.g. multiple sprints) and the higher number of repeated stimuli compared to running, as NO production is induced by hypoxia and muscle contractions (4,7). These results suggest that the specific movement dynamics and energy metabolism used in sports may affect baseline blood NO levels.

In this study, no significant relationship was found between blood NO levels and any anaerobic (vertical jump, T test) or aerobic power (IR1, LES and HRRS) parameter in both groups. Although a relationship between baseline NO levels and aerobic capacity of players is expected to be found in the study, the absence of this relationship may be due to players' experience and fitness level, as Nikolaidis et al (36) showed that elite volleyball players had higher levels of aerobic capacity compared with non-elite young players. Similarly, a positive relationship was found between baseline NO and peak power in the swimmers, but not in the volleyball players (34). The lack of relationship between the given anaerobic power performance parameters and NO levels in volleyball players, besides predominantly the anaerobic nature of volleyball, may be due to the fact that the physiological tests were not standardized and that they were carried out in different designs.

Significant relationships were observed between aerobic fitness and La elimination (3), HR recovery (37), and NO levels (33). Thus, in athletes with greater vasodilator reserve dependent on endothelium, lactate is expected to be metabolized faster. However, the fact that volleyball players do not possess higher aerobic capacity than endurance athletes, and matches being played at a physiological stress level under the 4.0 mM lactate threshold may play a role in the absence of a relationship between LES and HRRS and NO.

## CONCLUSION

Although volleyball matches created moderate physiological and OS levels in both groups, the increase of serum NO levels only in the player group may be related to a higher vasodilator potential and antioxidant capacity obtained by training adaptation. There was no relationship between blood NO levels and performance parameters which may be due to the anaerobic nature of volleyball. A further study on elite volleyball players during a real volleyball match may provide more extensive knowledge related to NO and OS for the sports science literature.

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