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Research Article / Araştırma Makalesi

The effects of handball and waterpolo training on blood nitric oxide and oxidative stress levels

Hentbol ve su topu antrenmanlarının kan nitrik oksit ve oksidatif stres düzeyleri üzerine etkileri

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ABSTRACT

Objective: Nitric oxide (NO) is a gas that has vasodilatory, antioxidant and metabolic regulatory properties. Both NO and oxidative stress (OS) are risk factors for atherosclerosis (AS). These properties of NO, therefore play a critical role in training adaptations. Handball and water polo are team sports requiring aerobic and anaerobic energy. However, since they are practised in different environments, their effects on blood NO and OS levels may be different. Therefore, this study aimed to compare the effects of these two team sports on blood NO and OS levels.

Material and Methods: Male handball players (HG, n=14), water polo players (WPG, n=12) and a control group (CG, n=13) of sedentary players participated in this study. Physical and physiological measurements, including critical velocity of the participants were made. The participants' fasting serum NO and OS indicators, and total antioxidant/oxidant status (TAS/TOS) were determined.

Results: TAS levels of HG and WPG were significantly lower than those of the control group ($p<0.05$, $p<0.01$), whereas their NO levels were higher than those of CG (19.5% and 25.7% respectively, $p>0.05$). No significant difference was present for NO between HG and WPG. A significant negative correlation was present between CV and serum NO level, and there was a positive correlation between maximal 1500 m running time and NO for handball players.

Conclusions: The findings of the study reveal that although training caused a significant decrease in antioxidant capacity in two team sports athletes, serum NO levels improved, thereby contributing to cardiovascular health. NO may harm aerobic endurance performance, but it had a beneficial effect on anaerobic power in the handball group, and none for water polo players.

Keywords: *Handball, water polo, nitric oxide, oxidative stress, critical velocitiy*

ÖZ

Giriş: Nitrik oksit (NO) vazodilatör, antioksidan ve metabolik regulatör özelliklere sahip bir gazdır. Hem NO hem de oksidatif stres (OS), ateroskleroz (AS) için birer risk faktörüdür. NO, bu özellikleri nedeniyle antrenman adaptasyonlarında önemli role sahiptir. Takım sporları olan hentbol ve sutopunun her ikisi de aerobik ve anaerobik enerji gerektirir. Ancak biri karada diğeri ise suda yapıldığı için kan NO ve OS düzeyleri üzerine etkileri farklı olabilir. Bu konu henüz açık olmadığı için, bu çalışmada bu iki takım sporunda antrenmanın kan NO ve OS düzeyleri üzerine etkilerinin karşılaştırılması amaçlandı.

Gereç ve Yöntemler: Bu çalışmaya erkek hentbolcu (HG, n=14) ve su topu oyuncusu (SG, n=12) ile sedanterlerden oluşan kontrol grubu (KG=13) katıldı. Katılımcıların kritik hız dahil fiziksel ve fizyolojik ölçümleri yapıldı. Katılımcıların açlık serum NO ve OS belirteçleri; total antioksidan/ oksidan statüleri (TAS/TOS) belirlendi. İstatistiksel anlamlılık için $p<0.05$ düzeyi kriter alındı.

Bulgular: HG ve SG'nin TAS düzeyleri kontrol grubuna göre anlamlı olarak daha düşük bulundu ($p<0.05$, $p<0.01$). NO düzeyleri ise kontrollere göre daha yüksekti (sırasıyla %19.5 ve %25.7, $p>0.05$). Ancak HG ve SG arasında NO için anlamlı bir farklılık saptanmadı. Hentbol grubunda, KH ile serum NO düzeyi arasında anlamlı negatif bir ilişki, maksimal 1500 m koşu zamanı ile NO arasında ise pozitif bir ilişki belirlendi.

Sonuç: Bu bulgular iki farklı takım sporu antrenmanlarının antioksidan kapasitesinde anlamlı bir düşmeye neden olmasına ragmen, serum NO düzeylerini yükselttiğini göstermektedir ve bu nedenle kardiyovasküler sağlığa da katkıda bulunabilir. NO'nun hentbol grubunda aerobik dayanıklılık performansı üzerinde negatif, anaerobik performans üzerinde ise yararlı etkisi olabilir. Sutopu sporcuları için ise böyle bir ilişki gözlenmedi.

Anahtar Sözcükler: *Hentbol, sutopu, nitrik oksit, oksidatif stres, kritik hız*

INTRODUCTION

Nitric oxide (NO) is a gas that has vasodilatory, metabolic regulator, and antioxidant properties (1). NO is produced endogenously from L-arginine and oxidized by nitric oxide synthase (NOS) enzyme to form citrulline and NO. It is an important determinant of blood pressure that inhibits platelet aggregation and vascular smooth muscle proliferation, and protects endothelial function against atherosclerosis (AS). Thus, NO is an independent risk factor for cardiovascular diseases (CVDs) (1). In addition, NO has the following functions: blood flow enhancement; oxygen, substrate, and

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hormone transport into the cell, regulation of glycolysis, mitochondrial respiration, and muscle contractile function (1,2). Aerobic exercise improves NO levels (1,3), but the effect in different team sports such as handball and water polo is not clear. It is crucial to know blood NO levels and to improve them through exercise.

NOS has at least three isoenzymes, one of which is located almost entirely in the vascular endothelium (eNOS) and secretes NO, which provides relaxation of blood vessels. The inducible NOS (iNOS) is produced in macrophages and many other cell types under inflammatory conditions. The last one is neuronal NOS (nNOS), found in the brain, the neuronal tissue, and tissues such as the skeletal muscle. eNOS and nNOS are more abundant in skeletal muscle (2). All isoforms of NO are transcriptionally regulated by hypoxia; thus, exercise such as water polo, which involves in partially hypoxic movements, may increase NO levels. nNOS expression increases with crush and severe injury, and with muscular activity, which may improve NO levels in handball. Handball is a fast-paced game, and contains many different movements, such as runs, jumping, and throwing, in a very short time (4). Water polo is played by swimming in a pool, which also requires high metabolic demands and explosive efforts in a short time (5), as in handball. Therefore, chronic training is expected to increase NO levels.

Another factor affecting NO levels is oxidative stress (OS). Exercise is a source of oxidative stress. If oxidant production exceeds the capacity of the antioxidant defense system, OS occurs (6-9). Therefore, increased free radicals can decrease NO concentrations (8), although they display antioxidant properties under normal physiological conditions. However, if superoxide radicals (SORs) increase, they react with NO to form a more potent oxidant, such as peroxynitrite ($ONOO^-$),and then the bioavailability and concentrations of NO decrease (1).

OS and antioxidant biomarkers can change throughout the season in both handball and water polo players (9,10). Therefore, special attention should be given to the ROS-induced reduction of NO bioavailability because ROS-induced mitochondrial dysfunction may lead to endothelial dysfunction and AS (1,11,12). Therefore, the different conditions existing in handball and water polo may change NO production and AS risk. In line with this view, significant benefits for athletes are present comparing with control groups for AS risk factors such as blood pressure, heart rate, and blood lipid and lipoprotein (BLLP) levels, which depend on the type of sports activity and training, and body position in handball and water polo players (13). However, no study was found, comparing blood OS and NO levels among players of these two sports. Therefore, we investigated the effects of training in male handball and water polo players on blood NO and OS levels, and the presence of NO-performance relationships.

MATERIALS and METHODS

Male handball players (HG, n=14), water polo players (WPG, n=12) and a control group (CG, n=13) of sedentary players voluntarily participated in the study.

Physical measurement methods: Height and body weight measurements were made on a scale. Body mass index (BMI) was obtained according to the formula: *BMI = weight (kg) / height (m)*^{2}.

Physiological measurements: Endurance measurements (as critical velocity, CV) of the athletes were made in the environment where the sport was performed: the pool for water polo, and the grass field for handball.

CV is the highest exercise load that can be sustained for a long time without getting tired (14). In water polo, maximum 200 m and 400 m free swimming times were taken in the pool, with 2-3 days intervals. In handball players and sedentaryplayers, CV was calculated based on the 800 m and 1500 m maximal running times again 2-3 days apart, according to the following formulas:

In water polo, *CV (m/s) = (400-200)/(400T-200T).* In handball, *CV (m/s) = (1500-800)/(1500T - 800T)*, where T is time in seconds. (14).

The subjects who voluntarily participated in the study were informed about the purpose of the study, its benefits, the tests to be performed, and the possible risks on a given day, and their written and signed consent was obtained through the "Voluntary Consent Form". Subjects were warned not to change much their diet, and not to perform heavy exercise at least two days before the measurements. Ethics Committee approval was approved by Ege University Faculty of Medicine Scientific Research Ethics Committee, with decision number 12-6/4.

Collection and analysis of blood samples: At least 3-4 days after the above tests, following a 12-hour night of fasting, blood samples were taken from the arm veins in the laboratory between 09-10 h in the morning. Serum tubes were centrifuged at 2000 × g for 15 min, and then incubated for 30 min at room temperature. Serum samples were kept at -20°C until the biochemical assays were performed in a single batch. Serum biochemical parameters were measured within a month.

Analysis of blood nitric oxide (NO) levels: Nitrite is the main product of NO oxidation in serum/plasma, and the concentration of nitrite accurately reflects changes in NOS activity (15). Therefore, in the present study, blood NO levels (as total nitrite), as nitrite in the sample and nitrite reduced from nitrate using cadmium (Cd^{++}) were assayed at 540 nm by using a nonenzymatic colorimetric kit (NB-88; Oxford Biomedical Research, Oxford, MI, USA) with the Griess reagent, as described by the kit manufacturer.

Serum nitric oxide (NO) analysis: NO measurements were performed spectrophotometrically (Shimadzu UV 1700, Japan) using a commercial kit (NB-88; Oxford Biomedical Research, Oxford, MI, USA) in the Exercise Biochemistry Laboratory of the Sports Science Faculty of the X university. The coefficients of variation (CVs) for the intra-assay and inter-assay of the NO kit were 6.8% and 7.9%, respectively. NO is given in mmol/l.

Determination of total antioxidant status (TAS) and total oxidant status (TOS): Serum TAS and TOS levels were determined spectrophotometrically using a commercial kit (Rel Assay Diagnostics, Türkiye) using a chromogenic method in an autoanalyzer (Abbott Architect C8000, USA) within one month. In the TAS method, antioxidant molecules in the serum create a new color with the chromogen substance used. The absorbance of this colored compound is proportional to the amount of antioxidants in the serum. TAS results are in mmol/l. Serum TOS levels were determined using a chromogenic method, spectrophotometrically. In this method, oxidant molecules in the serum create a new color with the chromogen substance used. The absorbance of this colored compound is proportional to the amount of oxidants in the serum. The results were given as equivalents of H_2O_2. The oxidative stress index (OSI) is the TOS/TAS ratio.

Blood serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels, which are accepted as classical coronary heart disease (CHD) risk factors were analyzed. Low-density lipoprotein cholesterol (LDL-C) levels were calculated according to the Friedewald (16) formula: *(LDL-C=TC-(TG/5)-HDL-C)*. People with a total cholesterol value >240 mg/dl and a triglyceride (TG) value >200 mg/dl were not present in the study. Classical CHD risk factors as TC/HDL-C, TC/LDL-C and TG /HDL-C ratios were obtained by calculating.

Statistical Analysis

Statistical analyses were performed with the SPSS 20 package program. The differences and correlations between the groups were analyzed nonparametrically. Whether a significant difference between the measurements obtained from the exercise and control groups was present was determined with the nonparametric Mann-Whitney U-Test. Spearman correlation analysis revealed the relationships between biochemical parameter levels and physical measurement scoresin both groups.

RESULTS

Physical and physiological parameters of the subjects are given in Table 1. The sport age of the water polo group (WPG)members was significantly greater than that of the handball group (HG) (p=0.047). The body height values of HG (p=0.032) and WPG (p=0.002) were considerably higher than the control group (CG). The body weights of the HG (p=0.004) and WPG (p=0.002) were again significantly higher than the CG. There was no significant differences between the groups for other physical and physiological parameters (p>0.05). No significant relationship was present between sports experience (sports age) and specified parameters. Although the CV value of HG was insignificantly higher than that of CG (5.3%), the CV value of WPG could not be compared with the others because they were performing in a different environment.

Table 1. Physical and physiological parameters of the subjects

Groups	HG (n=14)	WPG (n=12)	CG (n=13)
Age (yrs)	26.5±7.2	25.7±6.0	22.6±3.1
Height (cm)	186.3±7.0[a]	190.6±4.8[a]	180.4±6.8
Weight (kg)	91.3±11.1[a]	95.3±13.0[a]	77.7±8.6
BMI (kg/m^2)	23.3±5.3	25.0±5.4	23.8±2.3
Sports age(yrs)	13.9±8.0	17.9±5.8[b]	-
CV (m/s)	3.56±0.26	1.26±0.06	3.38±0.35

HG: handball group, WP: water polo group, CG: control group; BMI: body mass index, CV: critical velocity. Data as mean±SD; **a**: p<0.05 with respect to CG, **b**: p<0.05 wrt HG.

Serum NO levels of the CG was lower than that of the HG (19.5%, p>0.05) and WPG (25.7%, p>0.05). In contrast, TAS in the HG and WPG were significantly lower than those in the CG (p=0.026, p=0.021). No significant difference was present between the athlete groups for NO and other oxidative stress (OS) parameters (p>0.05) (Table 2). Serum TG (triglyceride) levels of the HG and CG were significantly higher than those of the WPG (p=0.024 and p=0.050). Serum TC/LDL-C ratio of WPG was significantly lower than that of CG (p=0.002) (Table 3).

Table 2. NO, oxidative stress and antioxidant parameters

Parameters	HG (n=14)	WPG (n=12)	CG (n=13)
NO (µmol/l)	65.4±19.2	68.8±23.6	54.7±8.1
TAS (µmol/l)	1.51±0.17[a]	1.51±0.12[a]	1.67±0.13[b]
TOS (µmol/l)	2.64±0.94	2.82±0.70	2.72±1.09
OSI (%)	1.76±0.62	1.87±0.48	1.63±0.62

HG: handball group, WPG: water polo group, CG: control group, NO: nitric oxide, TAS: total antioxidant status, TOS: total oxidant status, OSI: oxidative stress index. Data as mean±SD; **a**: p<0.05 wrt CG. **b**: p<0.05 wrt HG and WP.

Table 3. Classical risk factors for CHD and comparison of the groups

Parameters/Groups	HG (n=14)	WPG (n=12)	CG (n=13)
TC (mg/dL)	186.2±38.5	178.4±33.8	174.1±32.1
LDL-C (mg/dL)	116.9±33.5	118.8±29.0	102.5±26.4
HDL-C (mg/dL)	47.8±11.0	44.4±5.1	47.9±8.4
TG (mg/dl)	107.9±41.3[a]	76.0±24.1[a,b]	118.3±70.0[b]
TC/HDL-C	4.03±0.95	4.02±0.64	3.78±1.15
TC/LDL-C	1.62±0.16	1.53±0.12[c]	1.74±0.24
TG/HDL-C	2.43±1.18	1.73±0.58	2.69±2.04

HG: handball group, WPG: water polo group, CG: control group; TC: total cholesterol, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, TG: triglyceride. Data as mean±SD; [a]: p<0.05 wrt CG, [b]: p<0.05 wrt HG, [c]: p<0.01 wrt CG.

Relevant Correlations within Groups

In the handball group, expected significant correlations were present between TOS and OSI (r=0.948, p<0.001). Relationships were also obtained between the maximal 1500 m running time and CV (r=-0.818, p<0.05) or NO (r=0.685, p<0.05), and between CV and NO (r=-0.673, p<0.05). A negative relationship was present between TAS and NO (r=-0.741, p<0.05).

In the water polo group, natural significant relationships were present between maximal free 200 m and free 400 mswimming times (r=0.91, p<0.001), as predominantly aerobic energy is required for both distances. However, a negative and significant correlation was found between maximal free 400 m swimming time and CV (r=-0.998, p<0.001).

In the control group, there were expected significant correlations between 1500 m time and CV (r=-0.997, p<0.001), similar to water polo. A significant relationship was also naturally present between the maximal 800 m and 1500 m running times (r=0.988, p<0.001).

DISCUSSION

There was no significant difference between the groups of athletes for serum NO levels. However, the serum NO level of CG was lower than that of HG (19.5%) and that of WPG (25.7%) (Table 2), which partially shows the beneficial effects of physical fitness on cardiovascular health. However, TAS levels of HG and WPG were significantly lower than those of the control group (p<0.05, p<0.01).

Djordjevic et al. (17) did not find a significant difference in blood nitrite (NO_2) levels between adolescent male handball players and the control group, unlike ours. Moriguchi et al. (18) observed a significant increase in urinary NO levels in healthy female athletes (between 19 and 21 years old, n=6) who participated in a 3-day handball team training program during exercise. In another study (19), basal serum nitric oxide (BSNO) levels in male swimmers were significantly higher than the control group but not different from those of volleyball players.

Possible reasons for changes in NO: The level of shear stress exerted by the blood on the vessel wall is the main determinant of endothelial nitric oxide synthase (eNOS) enzyme production; thus, the high intensity of repetitive stimuli during training may be the source of NO (1). Thus, the observed NO differences may have occurred due to the increased NOS enzyme activity in the study. However, movements, temperature, and hydrostatic pressure in the water in water polo can limit breathing frequency and capacity, which may affect energy metabolism. Despite this, and the environmental differences and conditions in water polo, there was no significant difference between the two athletic groups for NO levels. Differences between studies may be primarily due to factors such as exercise type, intensity, duration, and study pattern.

A significant increase in plasma OS biomarkers and antioxidant enzymes in handball and water polo players was present (20-22). In addition, OS and antioxidant biomarkers change throughout the season in competitive athlete training (9,10). However, while moderate-intensity aerobic training has significant beneficial effects on the bioactivity of NO, and antioxidant capacity, chronic exercise at much higher intensity negatively affect NO bioactivity and antioxidant capacity (23).

Findings reveal that OS cannot be prevented in mixed type sports such as handball and water polo, despite increased antioxidant defence, unlike in the present study. However, ROS production during exercise is associated with adaptation processes, including antioxidant enzymes, and the induction of ROS-related molecular movements, including redox-sensitive transcription (24). OS and free radical production generally depend on exercise intensity (7,8). TOS and OSI levels did not significantly change, but the decrease in the antioxidant capacity (TAS) may be due to the increased OS during training in both teams in this study, without causing a significant decrease in NO levels.

Unlike the present study, significant differences in favor of athletes between blood pressure, heart rate, and blood lipid and lipoprotein levels in handball and water polo players were determined (13). In our study, serum triglyceride levels ofthe HG and CG were significantly higher than those of the WPG (p=0.024 and p=0.050), while the serum TC/LDL-C ratio of the WPG was significantly lower than that of the CG (p=0.002) (Table 3). In another study, endothelial function in 6-month center-based land walking tended to be superior to water walking in older sedentary individuals (25). Explaining the differences between studies is not possible with the present data. Further studies designed accordingly may be needed to clarify this.

Relationship between NO and performance: Plasma nitrite levels predict exercise capacity and may limit exercise capacity. Moreover, physical fitness and NO formation at rest are related, which may help explain the beneficial effects of physical exercise on cardiovascular health (26), unlike this study.

An unexpected negative relationship was present between NO and CV or TAS in handball. Nevertheless, a positive correlation was present between maximal 1500 m running time and NO in handball players. Similarly, positive correlation was found between BSNO and Wingate test peak power in swimmers, but not in the control and volleyball groups (20). NO inhibits the use of oxygen in the mitochondria (1), so a possible reason may be such a restriction. Furthermore, another reason for the negative associations of NO with CV and TAS may be the formation of more oxidant peroxynitrite (NO-derived radical substances, RONS) due to increased ROS. Marin et al. (21) had found a significant increase in RONS production with postgame antioxidant enzymes, which may support our view. These results may partially explain the relationships between the CV and NO or 1500 m running time, requiring further studies for betterexplanation. The small group samples and the uncontrolled daily diets of participants were also limits to our study.

CONCLUSION

Findings of the study reveal that, although training in two team sports caused a significant decrease in antioxidant capacity, serum NO levels improved, thereby contributing to cardiovascular health. NO may harm aerobic endurance performance, but a beneficial effect on anaerobic power was observed for handballers, but not for water polo athletes.

Ethics Committee Approval / Etik Komite Onayı

This study was approved by the Ege University Faculty of Medicine Scientific Research Ethics Committee (approval number 12-6/4, date: 20.04.2012).

Conflict of Interest / Çıkar Çatışması

The authors declared no conflicts of interest with respect to authorship and/or publication of the article.

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Author Contributions / Yazar Katkıları

Concept: XX; Design: XX; XXpervision: XX; Materials: XX; Data Collection and Processing: XX, XX; Analysis and Interprepation: XX; Literature Review: XX; Writing Manuscript: XX; Critical Reviews: XX.

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