

The Effects of Chronic Aerobic and Anaerobic Exercise on Blood Nitric Oxide Levels

Uzun Süreli Aerobik ve Anaerobik Egzersizlerin Kan Nitrik Oksit Seviyeleri Üzerine Etkisi

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ABSTRACT Objective: Nitric oxide (NO) is a gas which has vasodilator, antioxidant and metabolic regulator features. The positive effect of aerobic exercise is well known on the production of NO in healthy men. However, the effects of chronic anaerobic exercise on blood NO levels remain unclear. The aim of the present study was to investigate the effects of both chronic aerobic and anaerobic exercise on basal serum nitric oxide (BSNO) levels, and the relationships between BSNO and some aerobic and anaerobic performance parameters. **Material and Methods:** Three groups participated in the present study, each of them was composed of 11 healthy men with similar physical characteristics. The groups consisted of volleyball players as the anaerobic group (AnG), swimmers as the aerobic group (AeG) with long-term exercise background and volunteers who did not exercise regularly as the control group (CG). BSNO (as total nitrite) analysis was determined in fasting venous blood by using Griess method. Mean power and peak power as the criteria of anaerobic performance were determined by using Wingate test, and lactate minimum speed (LMS) was determined as the criterion of aerobic endurance with the LMS test based on lactate elimination. Finger tip lactate measurements were taken during certain segments of LMS test. **Results:** The BSNO value of the AeG was significantly higher compared to the CG (90.34 vs. 74.39 μ M), but it was not different from that of the AnG (80.02 μ M). No significant relationships were observed between the BSNO and LMS values in any group. The LMS value of the AeG was significantly greater than that in the CG (11.59 vs. 10.27 km/h). Peak power (13.11 vs. 9.84 w/kg) and mean power (8.55 vs. 7.36 w/kg) were significantly greater in the AnG compared to the AeG. A positive correlation was found between BSNO in the AeG and peak power ($r=0.648$, $p=0.031$) in the AeG. **Conclusion:** Based on the results of the present study, it is suggested that regular aerobic exercise may improve blood NO levels while anaerobic exercise does not; nevertheless, NO may play a role in both aerobic and anaerobic adaptations to exercise.

Key Words: Nitric oxid; lactic acid; exercise; anaerobic threshold

ÖZET Amaç: Nitrik oksit (NO) vazodilaör, antioksidan ve metabolik özelliklere sahip bir gazdır. Aerobik egzersizin kan NO düzeylerini iyileştirdiği bilinmektedir. Ancak anaerobik egzersizin kan NO düzeyleri üzerindeki etkisi belirsizdir. Bu çalışmanın amacı; aerobik ve anaerobik egzersiz antrenmanlarının kan NO düzeyleri üzerindeki kronik etkilerinin ve bazal serum NO (BSNO) düzeyi ile bazı aerobik ve anaerobik performans parametreleri arasındaki ilişkilerin araştırılmasıdır. **Gereç ve Yöntemler:** Çalışmaya benzer fiziksel özelliklere ve uzun bir antrenman geçmişine sahip üç grup katılmış olup, grupların herbiri sağlıklı 11 erkek gönüllüden oluşmaktadır. Anaerobik grup olarak (AnG) voleybol, aerobik grup olarak (AeG) yüzme branşına ait antrene sporcular ve kontrol grubu (KG) ise düzenli egzersiz yapmayan gönüllülerden oluşturuldu. Aerobik dayanıklılık performansının kriteri olarak laktat minimum hızı (LMH) LMH testi ile, anaerobik performans parametreleri (ortalama ve pik güç vb.) ise Wingate testi ile belirlendi. BSNO düzeyleri açlık venöz kanlardan Griess metodu ile belirlendi. LMH testi laktat eliminasyonuna dayanır bu nedenle; LMH testinin belirli kademelerinde parmak ucu kanlarından laktat ölçümleri gerçekleştirildi. **Bulgular:** Aerobik grubun BSNO değeri (90,34 μ M) kontrol grubununkinden (74,39 μ M) anlamlı olarak daha büyüktü, ancak anaerobik grubunkinden (80,02 μ M) anlamlı olarak büyük değildi. Aerobik grubun LMS değeri (11,59 km/s), kontrol grubuna göre (10,27 km/s) anlamlı olarak daha büyüktü. Anaerobik grubun zirve güç ve ortalama güç değerleri (sırasıyla 13,11 ve 8,55 w/kg) aerobik grubunkilere göre (9,84 ve 7,36 w/kg) anlamlı olarak daha büyüktü. Sadece aerobik grupta BSNO değeri ile zirve güç arasında pozitif korelasyon bulundu ($r=0,648$, $p=0,031$). **Sonuç:** Bu bulgular; düzenli aerobik egzersizin BSNO düzeylerini iyileştirebileceğini, anaerobik egzersizin ise bunda yeterli olmadığını, bununla birlikte NO'nun aerobik ve anaerobik egzersiz adaptasyonlarında rol oynayabileceğini gösterir.

Anahtar Kelimeler: Nitrik oksit; laktik asit; egzersiz; anaerobik eşik

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Nitric oxide (NO) is produced endogenously from a number of sources such as vascular endothelium and skeletal muscle in the body using the nitric oxide synthase enzymes (NOS) as a catalyst. The primary source of NO is L-arginine in mammals. There are three isoforms; endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). eNOS is active primarily in the endothelial tissue of blood vessels, where NO mediates vasodilation and relaxation of soft tissue. eNOS is a constitutively active isoform that produces low levels of NO at a steady rate over long periods to achieve its functional roles. nNOS is active primarily in the central and peripheral neurons where NO serves as an important neurotransmitter. Similar to eNOS, nNOS is constitutively active and produces low levels of NO over long periods. iNOS is active primarily in immune cells and glial cells, and is activated by pathogen recognition and cytokine release.

NO is important for both coronary and peripheral hemodynamic control and metabolic regulation during the performance of exercise. Skeletal muscle-derived NO is an important regulator of muscle contraction and metabolism.^{1,2} Exercise training in healthy individuals promotes adaptations in various NO systems, which can increase NO bioavailability through a variety of mechanisms, including increased NOS enzyme expression and activity. Such adaptations are likely to contribute to the increase in exercise capacity and the protection from cardiovascular events.¹ An enhanced inactivation and/or reduced synthesis of NO is seen in conjunction with risk factors for cardiovascular disease. This condition is referred to as endothelial dysfunction.

An increased production and/or impaired inactivation of reactive oxygen species (ROS), i.e., oxidative stress, leads to reduced bioactivity of NO. Cardiovascular risk factors, including hypercholesterolemia, hypertension, diabetes and smoking, as well as established diseases are associated with impairment of various NO systems.³ Exercise has particular efficacy in restoring dysfunction of the vascular endothelial NO system and exercise training in individuals with elevated cardiovascular risk or established disease can increase NO bioavail-

ability, and may represent an important mechanism by which exercise training provides benefit in the setting of secondary prevention.^{1,3}

Nitrite is the main oxidation product of NO in plasma and sensitively reflects acute and chronic changes in eNOS activity in healthy fasting volunteers. It has been shown that most of the circulating plasma nitrite is derived from eNOS activity in humans and other mammals.⁴ In cardiovascular system, nitrite can be reduced under hypoxic conditions to NO via its reaction with deoxyhemoglobin and deoxymyoglobin, regulating vascular tone and myocardial function.⁵

It is reported that exercise stress increases plasma nitrite concentration in healthy subjects; the increase in nitrite is NOS-dependent; the relative increase in nitrite correlates with endothelial function, and post-exercise nitrite concentration and age are independent predictors of stress duration and power. Nitric oxide synthase-derived plasma nitrite predicts exercise capacity, and impaired increase in plasma nitrite may limit exercise capacity. These results suggest a role for plasma nitrite in the adaptation of hemodynamics during exercise. Physical fitness and formation of NO at rest are positively linked to each other. This positive relation may help to explain the beneficial effects of physical exercise on cardiovascular health.⁶

The basal release of endothelium-derived NO is increased with 4 weeks of home based training in hypercholesterolemic patients, independently of lipid profile modification. This may contribute to the cardiovascular protective effects of exercise training, including reduced blood pressure, which may show that exercise conveys benefit beyond lipid profile modification via elevation in basal NO production.⁷

It was found that only moderate-intensity aerobic exercise augments endothelium-dependent vasodilation through the increased production of nitric oxide, but not mild or high and that high-intensity exercise possibly increases oxidative stress.⁸ In another study, it has been shown that long term different training strategies establish different basal nitrites and lipid peroxidation

levels in sportsmen. However, progressive exercise does not influence basal nitrite and the level of oxidative stress parameters neither at maximal load nor during the first 10 min of recovery in the sportsmen studied. For example, nitrite concentrations in rest were the lowest in taekwondo fighters, while rowers had the highest levels among the examined groups.⁹

The extent of oxidative stress induced by acute bout of exercise depends on many factors, as well as, exercise mode, intensity and duration, because different modes of exercise differ in energy demands, oxygen consumption and mechanical stress to tissues.¹⁰

Therefore, exercise intensity is clinically also quite important to select the appropriate kind of exercise, because an intense exercise can be hazardous to human vessels. However it is unclear how chronic anaerobic exercise like volleyball affects BSNO levels in healthy young men.

The lactate minimum speed (LMS) has been considered to be a hallmark of aerobic fitness,¹¹ LMS test is based on lactate elimination.¹² Lactate must be removed from the blood after intense exercise. In addition to inactive and active muscles, the liver and heart eliminate lactate during exercise.^{13,14}

Some studies have demonstrated that L-arginine, a precursor of nitric oxide, lowers lactate values in humans during submaximal exercise, while a rise in NO production increases work capacity.^{15,16} Long distance runners, cyclists, swimmers and triathletes have higher lactate threshold values, whereas in exercise types which apply high work loads on a short-term basis, lactate threshold values are low.¹⁷

Trained endurance athletes, who have greater endothelium-dependent vasodilator reserves, are expected to metabolize lactate more rapidly than the anaerobically trained athletes.¹ Thus, the LMS may be higher in aerobically trained compared to anaerobically trained athletes. Physiological needs and activities in aerobic and anaerobic exercise are different.

It is reported that NO may play a role in the adaptations that occur with training.¹ Hence, the

role of NO in aerobic and anaerobic exercise adaptations may be different. For example, it has been found that there are relationships between aerobic power ($\dot{V}O_2\text{max}$) and BSNO levels and aerobic exercise increases basal blood NO levels.^{1,18,19} However, the relationships between anaerobic exercise and BSNO remain largely unclear.

Furthermore, a correlation was found between BSNO production and the subjects' training history. It was shown that the athletes who trained professionally more than 3 years without longer cessations in the training process (more than 6 weeks) had greater basal production of NO₂ compared to subjects who trained less or had training cessations. Therefore long-term training may induce the increase in NO bioavailability, whilst training cessations, i.e. physical inactivity, have a negative effect.¹⁹ Thus, training history may also modify the effects of chronic exercise training on BSNO levels and the relationships between BSNO and anaerobic and aerobic performance parameters in volleyball players and swimmers with long-term exercise background.

Therefore, the aim of the present study was to investigate the chronic effects of aerobic and anaerobic exercise on BSNO levels, and the relationships between BSNO and aerobic (LMS) and anaerobic performance parameters (as peak power and mean power) in volleyball players as an anaerobic group and swimmers as an aerobic group.

MATERIAL AND METHODS

PARTICIPANTS

Thirty three healthy subjects with similar physical characteristics voluntarily enrolled in the study. Of the 33 participants, 11 male athletes (volleyball players; average age 21.7 ± 1.1 years) were chosen as the anaerobic group (AnG), 11 male athletes (swimmers; average age 21 ± 1.4 years) as the aerobic group (AeG), and 11 males (average age 21.7 ± 1 years) who had not exercised regularly for at least 3 months served as the control group (CG) (Table 1). On average, the anaerobic and aerobic groups respectively had 10 and 12 years of athletic background with all athletes in current training.

TABLE 1: Physical characteristics and comparison of the groups (n=11).

Variables	1	2	3	p	Groups comparison	p (groups)
Age (years)	21.00±1.41	21.72±1.19	21.72±1.00	0.284	1-2	0.171
					1-3	0.171
					2-3	1.000
Height (cm)	178.45±7.04	185.36±5.62	182.27±6.54	0.048	1-2	0.017
					1-3	0.174
					2-3	0.269
Body mass (kg)	75.54±9.82	79.10±6.74	77.27±6.26	0.570	1-2	0.293
					1-3	0.606
					2-3	0.587
BMI (kg/m ²)	23.73±2.99	23.01±1.67	23.26±1.55	0.735	1-2	0.443
					1-3	0.615
					2-3	0.790

BMI: Body mass index; 1: Aerobic group; 2: Anaerobic group; 3: Control group.

All subjects underwent an anamnesis for health history for control of anemia and acute infections. Those considered healthy according to the results were included in the study. Using this inclusion criterion, at least 60 people were screened to find suitable individuals for the study. On a specified date, participating volunteers were informed of the purpose and benefits of the study, including the tests that would be performed and potential risks. University School of Medicine’s Ethics Committee approved the study and the subjects provided written consent to participate in the study. The subjects were cautioned to maintain a stable diet for at least one week prior to the initial meas-

urements and to refrain from intense exercise for at least two days in advance of testing.

STUDY DESIGN

Physiological tests performed for all volunteers included the LMS test as an indicator of aerobic endurance, the Wingate test of anaerobic power, and physical measurements. NO concentrations in fasting venous blood were determined using the Griess Method, with cadmium as the reducing agent. BSNO measurements were performed two days before the Wingate test, and lactate measurements were performed before and after the Wingate test and in the intervening 1 min rest period between

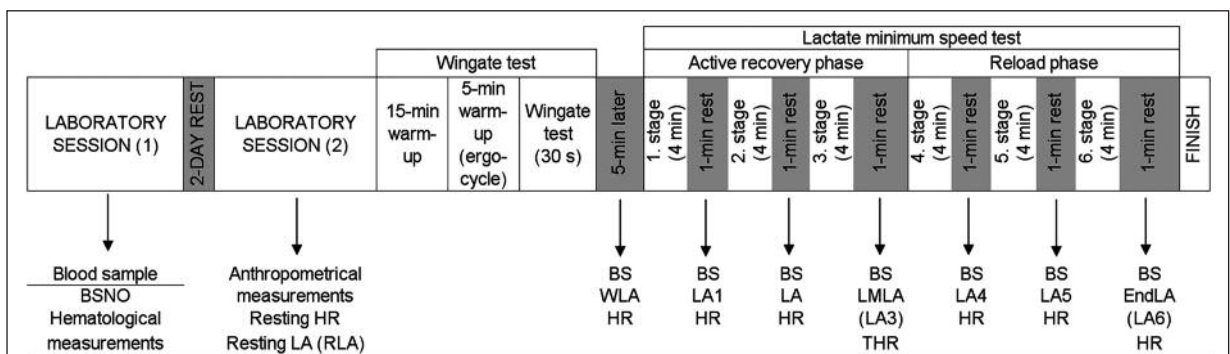


FIGURE 1: Study design.

Basal serum nitric oxide and hematological measurements were performed from fasting venous blood samples in the two day rest before the Wingate test, and lactic acid measurements were performed before and after the Wingate test and at various stages of the lactate minimum speed test. BSNO: Basal serum nitric oxide; HR: Heart rate; LA: Lactate; RLA: Resting lactate; BS: Blood sample; WLA: Lactate after Wingate test; LMLA: Lactate at lactate minimum point; THR: Threshold heart rate; EndLA: Lactate at the end of the test.

each 4 min period of running during the LMS test (Figure 1).

COLLECTION, PRESERVATION, AND ANALYSIS OF BLOOD SAMPLES

Two days prior to the exercise test, the subjects arrived at the laboratory at 8:30 am following at least 12 h of fasting, and initially rested sitting down for about 10 min. Without over-tightening the tourniquet, 10.0 mL of venous blood was then collected in a Vacutainer tube from an arm vein. Blood in the 10.0 mL tube was held at room temperature for 30 min and then centrifuged for 15 min at 1500 g. The acquired serum was distributed into plastic-capped Eppendorf tubes and transferred to a freezer at -80 °C. Nitric oxide (total nitrite) measurements were performed on these samples within 10-15 days. As indicated in the protocol,¹⁵ subjects returned to the laboratory 2 days later for the LMS test. At certain stages during the LMS test, 3-4 tubes of blood collected in capillary tubes containing heparin were transferred into lactate protective tubes and mixed, and were kept refrigerated until analysis.

NITRIC OXIDE ANALYSIS

NO has a half-life of a few seconds and in blood and soon oxidizes into nitrite containing vasoinactive and stable metabolites. Nitrite in whole blood is quickly transformed into nitrate. Therefore, the stable metabolites of NO, nitrite and nitrate, are measured to analyze blood NO.^{1,18}

Plasma nitric oxide analyses for the study were performed using Oxis kits (Oxis International Inc., Beverly Hills, CA, USA). The method is based on spectrophotometric determination of the absorbance of pink azo dye derived from exposure of nitrite to the Griess reagent. Nitrite (NO₂), on the other hand, is generated by reduction of nitrate (NO₃) the basic metabolite of nitric oxide using cadmium (Cd²⁺). Using this method, nitrite inherent in the sample and nitrite reduced from nitrate were measured cumulatively. Thus, total nitrite levels were used in the current study as the representative of the BSNO.

LACTATE ANALYSES

To perform the lactic acid analysis, the subject's fingertip was first wiped with alcohol and then

dried with a piece of cotton wool. The fingertip was then pierced with a lancet and squeezed gently. For each subject, blood was collected in two hematocrit capillary tubes with heparin, and later transferred into blood lactate-preserving tubes. Samples were stored in a refrigerator until the analysis commenced. Lactate samples were analyzed on the same day using a YSI 1500 sport lactate analyzer (YSI, Inc., Yellow Springs, Ohio, USA). Daily calibration of the analyzer was carried out with 5 and 15 mM standard lactate calibration solutions. The 5 mM standard calibration solution was used for analyzer calibration prior to measuring each subject's samples. A lactate membrane, a tampon to prepare the reactive substance, and erythrocyte-breaking lysing agent were used for measurements with the device.

WINGATE ANAEROBIC POWER AND CAPACITY TEST

A 30-s Wingate anaerobic cycle test was used with model 824E Monark cycle ergometer (Monark, Stockholm, Sweden). Following a general warm-up limited to 15 minutes, subjects performed a 5 min long special warm-up exercise in laboratory conditions on another Monark Ergometer cycle involving 2-3 sets of 2-3 second long maximal speed pedaling. After the warm-up, subjects used the cycle ergometer dedicated for measurement and pedaled at supramaximal speed for 30 seconds against a load equivalent to 75 g/kg body mass. Pedaling speed in the first 5 seconds and the power calculated from the load gave peak power (W). Mean power was calculated as the overall pedaling speed for 30 seconds and the power calculated from the load.²⁰ Peak power and mean power were determined using an online data-acquisition system (Monark Wingate Ergometer Test, Monark Body Guard, AB, Version 1.0).

LACTATE MINIMUM SPEED

The LMS test was performed for the first time to predict running speed at maximal lactate steady state.¹¹ The LMS test begins with intense exercise resulting in metabolic acidosis. A 5-min rest period follows the exercise period to allow for a rise in lactate. Later, a standard anaerobic threshold test is

initiated with blood lactate collected at each step. Under these conditions, the LMS test begins with high lactate values (~8 mM) and produces a U-shaped blood lactate curve during the test (Figure 2). Blood lactate removal begins at the start of the lactate minimum test. The lactate concentration continues falling until an aerobic-anaerobic transition balance is reached. The decline in blood lactate values is termed the active recovery phase. The reduction ends once a balance is reached and the lactate concentration begins to rise again because the test is still in progress (the speed is rising). The subsequent rise in the blood lactate value is termed the reloading phase. The point of inflection is termed the lactate minimum (LM), also known as the aerobic-anaerobic balance and maximal lactate threshold value.^{11,12,21,22} An advantage of the LMS test is that it is a single test that can determine both anaerobic power and the anaerobic threshold. However, the test is not suitable for all individuals as it involves short-term, high-intensity loads.²³

In accordance with the protocol, the subjects underwent 3 acute exercise-loading phases during the LMS test.²⁴ The first phase was a supramaximal Wingate cycle exercise test. Following the exercise phases, the subjects had 5-min passive resting periods. Blood lactate was analyzed to determine the degree of metabolic acidosis after 5 min.

The initial speed for the active recovery phase of the LMS test was taken in the present study as 70% of the time each athlete runs 3000 meters. The speed increase for each step was set to 0.5-1 km/h. This step took place on the treadmill to

buffer metabolic acidosis occurring after maximal exercise and to eliminate rising lactate values. This phase was carried out in the form of 3 different submaximal intermittent treadmill tests that intensified gradually and lasted 4 min for each step. This phase encompassed the balance point between lactate production and elimination and continued until the lactate minimum point. The blood lactate value at this point was termed lactate at the lactate minimum point (LMLA).

This phase was followed by a reloading phase that continued until the maximal level was reached, in the form of three gradually increasing speeds each lasting four minutes. The load increases continued until the subjects were exhausted; the majority of the subjects ended the test at step 6. The subjects' heart rates at the end of the test reached or exceeded age predicted maximum heart rates. At the end of the test, the exercise load at the minimum lactate level was considered to be the LMS.²⁴

HEART RATE MEASUREMENTS

A heart rate monitor (RS 400; Polar Electro Oy, Kempele, Finland) was used to continuously measure heart rate during the tests. Real-time heart rate data were sent to the monitor from the chest strap by radio waves.

STATISTICAL ANALYSES

The data are presented as means±standard deviations. The Kolmogorov-Smirnov test was used to determine whether the data were normally distributed. Levene's test was employed for homogeneity of the variances of the groups. The normality and homogeneity tests determined that the data were normally distributed and the variances of the groups were homogeneous. Thus, parametric analysis methods were used. Relationships between blood NO levels and LMS, peak power, mean power, minimum power and fatigue index were investigated using correlation analysis (Pearson correlation coefficient). One-way analysis of variance (ANOVA) and the post-hoc least significant difference (LSD) test were used to compare average differences between the exercise and control groups. SPSS 11.0 (SPSS Inc., Chicago, IL, USA) was

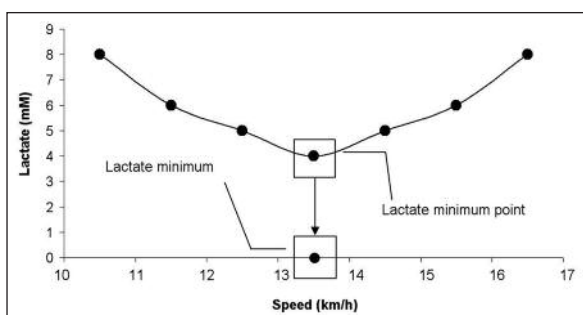


FIGURE 2: The lactate minimum point.

The point of inflection is termed the lactate minimum (LM), also known as the aerobic-anaerobic balance and maximal lactate threshold value.^{11,21,22,24}

used analyze the data. Statistical significance was defined as $p < 0.05$.

RESULTS

There was a significant difference in BSNO levels between the AeG ($90.34 \pm 21.57 \mu\text{M}$) and the CG ($74.39 \pm 14.03 \mu\text{M}$) ($p = 0.048$). However, there was no difference between the AnG ($80.02 \pm 20.98 \mu\text{M}$) and the CG nor between the AnG and the AeG.

Significant differences for peak power output were found between all three groups. AnG and CG values for minimum power were also significantly greater than those of the AeG (Table 2). Fatigue index (FI) value ($52 \pm 2.9\%$) for the AnG was significantly greater than that of the AeG ($47 \pm 8.5\%$) ($p = 0.030$). AeG LMS ($11.59 \pm 1.17 \text{ km/h}$) value was significantly greater than that of the CG ($10.27 \pm 0.68 \text{ km/h}$) ($p = 0.003$), however there was no difference between the AnG ($10.95 \pm 0.96 \text{ km/h}$) and the AeG nor between the AnG and the CG (Table 2).

A positive relationship was found between BSNO levels and peak power ($r = 0.648$, $p = 0.031$) in the AeG, but not for other measures of anaerobic power in the AeG nor for any of the anaerobic performance criteria and BSNO values for the other groups.

There was no relationship between BSNO and LMS for any group ($r = 0.001/p = 0.998$ in AeG, $r = 0.035/p = 0.919$ in AnG, $r = -0.241/p = 0.476$ in CG).

DISCUSSION

The results showed that the BSNO value of the AeG was significantly higher than that of the CG, but not significantly different from that of the AnG. The BSNO level of the AnG was also 7.1% greater than that of the control group, but not significantly. In addition, a positive relationship was found between the BSNO and the peak power in the AeG, but no significant relationships were found between anaerobic (Wingate) performance parameters and BSNO values in the other groups. A

TABLE 2: Anaerobic and aerobic performance data and comparison of the groups (n=11).

Variables	1	2	3	p	Groups comparison	p (groups)
Peak power (W/kg)	9.84±1.43	13.11±0.96	11.62±1.22	0.000	1-2	0.000
					1-3	0.002
					2-3	0.009
Mean power (W/kg)	7.36±0.84	8.55±0.43	8.16±0.86	0.003	1-2	0.001
					1-3	0.018
					2-3	0.219
Minimum power (W/kg)	5.11±0.63	5.91±0.91	5.85±0.88	0.035	1-2	0.030
					1-3	0.041
					2-3	0.883
FI (%)	47±8.5	52±2.9	51±8.4	0.047	1-2	0.030
					1-3	0.326
					2-3	0.210
Lactate (mM)	4.38±0.78	4.66±0.67	4.63±0.62	0.458	1-2	0.261
					1-3	0.307
					2-3	0.915
LMS (km/h)	11.59±1.17	10.95±0.96	10.27±0.68	0.012	1-2	0.132
					1-3	0.003
					2-3	0.107
Threshold heart rate (beat/min)	171.25±7.28	169.36±5.21	170.90±5.24	0.795	1-2	0.382
					1-3	0.888
					2-3	0.462

FI: Fatigue index; LMS: Lactate minimum speed; 1: Aerobic group; 2: Anaerobic group; 3: Control group.

previous study also found that BSNO levels of both young and older athletes involved in regular aerobic exercise were significantly greater than that in the control group, consistent with the present study results. The same study also suggested a relationship between greater microcirculatory function and maximal oxygen consumption ($VO_2\text{max}$) potentially due to increased NO levels.²⁵

Another study conducted on healthy groups with different fitness levels determined that physical fitness levels and resting NO production were positively related and the plasma nitrate levels of athletes were significantly greater than those of sedentary individuals,⁶ which is consistent with the present study results. However, no significant relationship was observed between the BSNO value and LMS in any group in the present study. NO plays a role in hemodynamic adaptations through exercise. It has been reported that post-exercise nitrite concentrations are related to exercise performance and a decline in nitrate levels can limit exercise capacity.²⁶

A study conducted on middle aged (n=32) people reported that after 12 weeks of aerobic exercise in the form of 30 min rapid walking (repeated 5-7 times per week), endothelium-dependent vasorelaxation in both hypertensive and normotensive individuals improved together with an increase in NO production.²⁷

It was found that, although not statistically significant, the NO levels of a group playing football for health were greater than groups either participating in jogging or living a sedentary life.¹⁸ Another study found that BSNO levels in professional male football players were significantly greater than those in the control group.²⁸ These differences were attributed to the greater anaerobic qualities 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.¹ Hence, these results suggest that the specific movement dynamics of football may affect NO levels. The movement dynamics and differences in energy metabolism between the volleyball group (considered the anaerobic group)

in the present study and the football group in the previous study may partially explain the differences between the findings of these studies.

All isoforms of NO can be regulated by transcription with hypoxia. nNOS expression increases with contusion, severe injury, and muscular activity. Vascular and muscle nNOS are upregulated with chronic exercise.^{1,29} It has been demonstrated that both fast and slow contracting fibers of skeletal muscle express both NOS proteins. However, nNOS levels are related to fast contracting, glycolytic muscles while eNOS levels are mostly related to oxidative muscles. Therefore, it is expected that muscles participating in aerobic or anaerobic exercise should produce different levels of NO in different sport activities such as swimming and volleyball.

In healthy subjects, only moderate-intensity aerobic exercise augments endothelium-dependent vasodilation through the increased production of nitric oxide, but not mild or high. A 12-week-period of exercise of high aerobic intensity (at 70-80% of $VO_2\text{max}$) increased the indices of oxidative stress, such as plasma concentration of 8-hydroxy-2-deoxyguanosine and serum concentration of malondialdehyde-modified low-density lipoprotein, and decreased endothelium-dependent vasodilation in healthy young men.⁸ It has been reported that oxidized LDL inhibit NO production, directly inactivating NO.¹ In another study, it has been shown that long term different training strategies establish different basal nitrites and lipid peroxidation levels in sportsmen. For example, nitrite concentrations in rest were the lowest in taekwondo fighters, while rowers had the highest levels among examined groups.⁹

It is thought that ROS are not produced excessively under physiological conditions in healthy subjects. It has been reported that the massive increase in oxygen uptake that occurs in skeletal muscle during exercise is associated with an increase in the generation of ROS (as superoxide).²⁷ Oxidant concentrations exceeding a certain limit inhibit the bioeffectiveness of NO.³⁰ The balance between inherent superoxide and NO levels plays

an important role in the normal endothelium in maintaining its function. Oxidative stress reduces the amount of bioactive NO by chemical inactivation to form toxic peroxynitrite. Peroxynitrite in turn can “uncouple” endothelial NO synthase to become a dysfunctional superoxide-generating enzyme that contributes to vascular oxidative stress.^{1,3}

It is thought that regular aerobic exercise increases nitric oxide production with up-regulation of eNOS gene expression and vascular endothelial growth factor (VEGF)-induced angiogenesis and decreases NO inactivation with augmented antioxidant system, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), and attenuation of nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate oxidase activity, leading to an increase in NO bioavailability.²⁷ It has been shown that biological antioxidative potential decreased after a 6-week training period in female volleyball players.³¹ Thus, long-term anaerobic training for activities such as volleyball can deteriorate endothelium-related vasodilation and antioxidant system. Therefore, these data may help to explain the reasons for lower serum NO levels in anaerobic volleyball group compared to aerobic swimming group.

It has been reported that a 10-day treadmill exercise program increases vascular eNOS gene expression, which in turn increases acetylcholine-induced NO production. Furthermore, it was noted that an increase in shear stress increases vascular structure and function, and induces histological changes.⁸ Shear stress occurring during physical exercise is an important factor in NO production. During exercise, shear stress increases in the vein endothelium, which results in vasodilation.^{32,33} Increased transport of blood and substrate to active muscles including the myocardium has a pronounced effect on exercise performance. Over the long term, this can result in metabolic enzyme changes and restructuring of veins.^{1,29} In addition to regulating vascular tone, NO plays an important role in maintaining a balance between vasodilators and constrictors. Although the mechanisms by which chronic aerobic exercise affects BSNO levels remain largely unknown, they may be related to

the reported positive effects of regular exercise, including blood pressure regulation, lipid and glucose metabolism, neuronal and neurohormonal factors, decrease in body weight and increases in shear stress.⁸ Similarly, the manner in which regular aerobic exercise has a healing effect on endothelium dysfunction remains largely unknown; however, it has been reported that acute and chronic exercise-induced endothelium-dependent vasodilatation results from an increase in NO production.⁸ All of the factors discussed above may play differentiating roles in the effects aerobic and anaerobic exercise have on BSNO levels and LMS.

Previous studies found significant relationships between blood NO levels and VO_2 max although no significant relationships between NO levels and LMS were found in the present study.^{6,18,19,25} Many factors may have influenced this outcome, including the study design, small number of subjects, and NO measurement methods. Nonetheless, LMS, a value representing aerobic endurance, and BSNO levels were found to be greater in the AeG than in the AnG and CG. It has been demonstrated that NO levels increase with exercise in animal studies, resulting in both capillarization and arteriogenesis.³⁴

The positive effects aerobic training has on the central and vagal nervous systems are well known. Athletes have a greater heart rate volume, a larger arteriovenous O_2 difference, and greater venous return, related to activation of the nervous system.¹ Endurance training also increases lactate clearance. This reflects increased hepatic capacity for gluconeogenesis as well as increased lactate transport capacity and oxidative capacity and reduced glycogenolysis in muscle. Therefore endurance performance can be predicted from the plasma lactate versus exercise intensity.³⁵ In consequence, higher LMS and BSNO levels observed in the present study for the AeG compared to the CG may indicate a potential interaction between LMS and BSNO.

The positive relationship observed between the anaerobic performance parameter (peak power) and BSNO in the AeG suggests that NO may also

play a role in anaerobic training adaptations. It is known that, independent of blood circulation, NO increases muscle glucose uptake and inhibits glycolysis and creatine phosphate depletion, thereby, preserving intracellular muscle energy reserves.¹ Therefore, it is interesting that we observed a positive relationship between NO levels and peak power in the aerobic group, though the reason for this relationship is not known. However, this relationship may be due to specific activity patterns in swimming as well as the volume and frequency of swim training, which are greater than those encountered in volleyball, which depends much more on anaerobic power. Thus, more research is required to understand the reason for this relationship.

CONCLUSION

This study investigated the effects of chronic aerobic and anaerobic exercise on blood NO levels as well as the relationship between NO levels and lactate minimum speed, as a criterion measure of aerobic endurance, together with Wingate performance parameters. However, the direct relationships among these parameters, except peak power, could not be determined. It is interesting that we

observed a positive relationship between NO and anaerobic peak power in the aerobic group. Future research may be carried out to explore the reasons for this relationship in sprint versus endurance trained men and women. The results suggest that regular aerobic exercise may improve blood NO levels, but not anaerobic exercise, nevertheless NO may play a role in both aerobic and anaerobic adaptations to exercise.

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REFERENCES

- Kingwell BA. Nitric oxide-mediated metabolic regulation during exercise: effects of training in health and cardiovascular disease. *FASEB J* 2000;14(12):1685-96.
- Stamler JS, Meissner G. Physiology of nitric oxide in skeletal muscle. *Physiol Rev* 2001; 81(1):209-37.
- Förstermann U. Nitric oxide and oxidative stress in vascular disease. *Pflugers Arch* 2010;459(6):923-39.
- Kleinbongard P, Dejam A, Lauer T, Rassaf T, Schindler A, Picker O, et al. Plasma nitrite reflects constitutive nitric oxide synthase activity in mammals. *Free Radic Biol Med* 2003;35(7): 790-6.
- Rassaf T, Lauer T, Heiss C, Balzer J, Mangold S, Leyendecker T, et al. Nitric oxide synthase-derived plasma nitrite predicts exercise capacity. *Br J Sports Med* 2007;41(10):669-73; discussion 673.
- Jungersten L, Ambring A, Wall B, Wennmalm A. Both physical fitness and acute exercise regulate nitric oxide formation in healthy humans. *J Appl Physiol* 1997;82(3):760-4.
- Lewis TV, Dart AM, Chin-Dusting JP, Kingwell BA. Exercise training increases basal nitric oxide production from the forearm in hypercholesterolemic patients. *Arterioscler Thromb Vasc Biol* 1999;19(11):2782-7.
- Goto C, Higashi Y, Kimura M, Noma K, Hara K, Nakagawa K, et al. Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: role of endothelium-dependent nitric oxide and oxidative stress. *Circulation* 2003;108(5):530-5.
- Cubriilo D, Djordjevic D, Zivkovic V, Djuric D, Blagojevic D, Spasic M, et al. Oxidative stress and nitrite dynamics under maximal load in elite athletes: relation to sport type. *Mol Cell Biochem* 2011;355(1-2):273-9.
- Fisher-Wellman K, Bloomer RJ. Acute exercise and oxidative stress: a 30 year history. *Dyn Med* 2009;8:1.
- Teghtur U, Busse MW, Braumann KM. Estimation of an individual equilibrium between lactate production and catabolism during exercise. *Med Sci Sports Exerc* 1993;25(5): 620-7.
- Simões HG, Grubert Campbell CS, Kokubun E, Denadai BS, Baldissera V. Blood glucose responses in humans mirror lactate responses for individual anaerobic threshold and for lactate minimum in track tests. *Eur J Appl Physiol Occup Physiol* 1999;80(1):34-40.
- Costill DL, Coyle E, Dalsky G, Evans W, Fink W, Hoopes D. Effects of elevated plasma FFA and insulin on muscle glycogen usage during exercise. *J Appl Physiol* 1977;43(4):695-9.
- Alemdaroğlu U, Koz M. [Recovery after exercise; types and methods of recovery]. *Turkiye Klinikleri J Sports Sci* 2011;3(1):38-46.
- Burtscher M, Brunner F, Faulhaber M, Hotter B, Likar R. The prolonged intake of L-arginine-L-aspartate reduces blood lactate accumulation and oxygen consumption during submaximal exercise. *J Sports Sci&Med* 2005;4(3):314-22.

16. Maxwell AJ, Ho HV, Le CQ, Lin PS, Bernstein D, Cooke JP. L-arginine enhances aerobic exercise capacity in association with augmented nitric oxide production. *J Appl Physiol* 2001;90(3):933-8.
17. Irmak R, Bediz CŞ, Aksu İ, Topçu A, Güleçer B. [Sensitivity of blood lactate threshold changes, determined by different mathematical methods in trained females and males]. *Türkiye Klinikleri J Sports Sci* 2011;3(1):1-7.
18. Turgay F, İşlekel H, Karamızrak SO, Yenisey Ç, Kocahan T, Selamoğlu S. The effect of two different healthy living activities/sports on serum nitric oxide levels in middle-aged males. *Turkish Journal of Sports Medicine* 2006; 41(2):105-12.
19. Djordjevic D, Jakovljevic V, Cubrilo D, Zlatkovic M, Zivkovic V, Djuric D. Coordination between nitric oxide and superoxide anion radical during progressive exercise in elite soccer players. *Open Biochem J* 2010;4:100-6.
20. Bediz CS, Gökbel H, Kara M, Uçok K, Cikrikçi E, Ergene N. Comparison of the aerobic contributions to Wingate anaerobic tests performed with two different loads. *J Sports Med Phys Fitness* 1998;38(1):30-4.
21. Carter H, Jones AM, Doust JH. Effect of incremental test protocol on the lactate minimum speed. *Med Sci Sports Exerc* 1999;31(6):837-45.
22. MacIntosh BR, Esau S, Svedahl K. The lactate minimum test for cycling: estimation of the maximal lactate steady state. *Can J Appl Physiol* 2002;27(3):232-49.
23. Svedahl K, MacIntosh BR. Anaerobic threshold: the concept and methods of measurement. *Can J Appl Physiol* 2003;28(2): 299-323.
24. Simões HG, Campbell CS, Kushnick MR, Nakamura A, Katsanos CS, Baldissera V, et al. Blood glucose threshold and the metabolic responses to incremental exercise tests with and without prior lactic acidosis induction. *Eur J Appl Physiol* 2003;89(6):603-11.
25. Franzoni F, Galetta F, Morizzo C, Lubrano V, Palombo C, Santoro G, et al. Effects of age and physical fitness on microcirculatory function. *Clin Sci (Lond)* 2004;106(3):329-35.
26. Rassaf T, Flögel U, Drexhage C, Hendgen-Cotta U, Kelm M, Schrader J. Nitrite reductase function of deoxymyoglobin: oxygen sensor and regulator of cardiac energetics and function. *Circ Res* 2007;100(12):1749-54.
27. Higashi Y, Yoshizumi M. Exercise and endothelial function: role of endothelium-derived nitric oxide and oxidative stress in healthy subjects and hypertensive patients. *Pharmacol Ther* 2004;102(1):87-96.
28. Dumlupınar C, Turgay F, Bereket SY, Çeçen AA. The relation between serum nitric oxide levels and maximal effort, and recovery blood lactate in professional football players. *Turkish Journal of Sports Medicine* 2006; 41(1):37-44.
29. Britten MB, Zeiher AM, Schächinger V. Clinical importance of coronary endothelial vasodilator dysfunction and therapeutic options. *J Intern Med* 1999;245(4):315-27.
30. Yoshida N, Ikeda H, Sugi K, Imaizumi T. Impaired endothelium-dependent and -independent vasodilation in young female athletes with exercise-associated amenorrhea. *Arterioscler Thromb Vasc Biol* 2006;26(1):231-2.
31. Martinović J, Dopsaj V, Kotur-Stevuljević J, Dopsaj M, Vujović A, Stefanović A, et al. Oxidative stress biomarker monitoring in elite women volleyball athletes during a 6-week training period. *J Strength Cond Res* 2011; 25(5):1360-7.
32. Kubota T, Imaizumi T, Oyama J, Ando S, Takeshita A. L-arginine increases exercise-induced vasodilation of the forearm in patients with heart failure. *Jpn Circ J* 1997;61(6):471-80.
33. Maxwell AJ, Schauble E, Bernstein D, Cooke JP. Limb blood flow during exercise is dependent on nitric oxide. *Circulation* 1998;98(4): 369-74.
34. Lloyd PG, Yang HT, Terjung RL. Arteriogenesis and angiogenesis in rat ischemic hindlimb: role of nitric oxide. *Am J Physiol Heart Circ Physiol* 2001;281(6):H2528-38.
35. Stalknecht B, Vissing J, Galbo H. Lactate production and clearance in exercise. Effects of training. A mini-review. *Scand J Med Sci Sports* 1998;8(3):127-31.