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Acute effect of speed exercise on nitric oxide (NO) level of footballers

Aim: Exercise is one of the low-cost and easiest ways for improving life standards and physical fitness, and reducing body stress. Nitric oxide (NO) is defined as the "surprising soldier mediator" of biological systems. NO is one of the relaxation factors coming from the vascular endothelium. This relaxation in smooth muscles leads to vasodilatation in veins. The purpose of this study is to evaluate the acute effect of 20-meter sprints of footballers on NO levels.

Materials and Methods: 22 footballers with an age range of 18–32 were voluntarily enrolled in the study. The heights and weights of the subjects were measured. Speed measures were taken with a photocell system. The 20-meter sprint was repeated for 10 times. Blood samples were taken before and immediately after the sprint. For statistical analysis paired t test was used.

Results: The mean age, height, and weight of the footballers included in the study were 24.21 ± 3.41 years, 173 ± 7.04 cm, and 67.85 ± 5.17 kg, respectively. The mean NO values were 21.45 ± 2.08 immediately after the exercise and 25.59 ± 1.59 before the exercise (mean ± SD). This decrease in the NO level after the exercise, compared to before, were not statistically meaningful (P = 0.052).

Conclusions: The fact that the exercise was short-term yet with maximal load and negative developments occurred within the body may have highlighted the oxidative damage. The NO defence used against this damage may have decreased the values.

Key Words: Nitric oxide, speed exercise, footballer

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Futbolcularda sürat egzersizinin nitrik oksit (NO) düzeyi üzerine akut etkisi

Amaç: Egzersiz, insanları stresten uzaklaştıran, hayat standartlarını yükselten ve fiziksel uygunluğu iyi duruma getirmek için kullanılan en ucuz ve kolay yoldur. Nitrik oksit (NO) biyolojik sistemlerin "şaşırtıcı asker aracısı" olarak tanımlanır. NO vasküler endotelyumdan çıkan dinlenme faktörüdür. Bu düz kaslardaki relaks damarlarla vasodilatasyona neden olur. Bu çalışmanın amacı; futbolcularda 20 metrelik sürat koşularının NO düzeylerine akut etkisini araştırmaktır.

Gereç ve Yöntem: Araştırmaya yaşları 18-32 yaş arasında değişen 22 futbolcu gönüllü olarak katıldı. Deneklerin boy ve beden ağırlık ölçümleri alındı. Sürat ölçümleri fotosel ile yapıldı. 20 metrelik sürat koşusu 10 kez tekrar ettirildi. Sürat egzersizi öncesi ve hemen sonrası kan örnekleri alındı. İstatistiksel analiz için paired t testi kullanıldı.

Bulgular: Çalışmaya dahil edilen futbolcuların yaşları 24,21 ± 3,41 yıl, boyları 173 ± 7,04 cm. ve beden ağırlığı 67,85 ± 5,17 kg. olarak belirlendi. Sürat egzersizi öncesi NO değerleri 25,59 ± 1,59' iken, egzersiz hemen sonrası 21,45 ± 2,08 olarak bulundu (A.O ± SS). Egzersizden hemen sonrasındaki NO düzeyindeki bu düşüş istatistiksel olarak anlamlı değildi (P = 0,052).

Sonuç: Egzersizin kısa süreli ancak maksimal yüklenmeli olması, vücutta meydana gelen negatif gelişmeler oksidatif hasarı öne çıkarmış olabilir. Bu hasara karşı kullanılan NO savunması, değerleri aşağıya düşürmüş olabilir.

Anahtar Sözcükler: Nitrik oksit (NO), sürat egzersizi, futbolcu

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Introduction

Until 15-20 years ago, NO was thought to be merely the exhaust gas of automobiles, an atmospheric gas found in cigarettes, and an agent polluting the atmosphere. It has been articulated in previous studies that NO, which has been qualified as a harmful molecule since it is a free radical due to the unpaired electron it bore, is actually an important molecule that balances the blood pressure and vasodilatation (1,2).

NO causes vasodilatation in veins and accelerates blood flow. NO, which regulates the blood circulation, also keeps the veins clean. The vein network rich in NO is slippery like Teflon and allows for the flow of plaques preventing the enlargement of clots, whereas the unhealthy vein, or the vein poor in NO, leads to collection of plaques in the adhesive inner surface of the vein (3,4).

NO radical prevents the excessive accumulation of free radicals in tissues by reacting with other free radicals (5,6). Whereas other free oxygen radicals are harmful in every concentration, NO plays a role in regulating a range of physiological events, such as digestion system, host defence and non-specific immunity. However, when generated inappropriately and excessively, it causes a lot of pathological conditions (7).

Sport generally has significant features like speed, strength, endurance, and flexibility. Speed is the ability to carry oneself from one point to another in the shortest time possible (8).

In sports trainings, the organism is exposed to loads which are over daily life levels. In exercise, it is blood that provides the metabolic and O_2 needs of the tissues. Blood pressure is the force that enables the blood flow. Blood pressure is the pressure that exerts the blood on the vein walls (9).

The benefits of exercising have been proven plenty of times. However, negative changes can also be expected in connection with the short-term and extreme changes that exercise may cause in the human body. However, exercise, if performed regularly and efficiently, helps strengthen the cardiovascular, hormonal, and immune system, and increase the muscle mass.

This study was conducted to analyze the acute effect of speed exercise on NO levels of footballers.

Materials and Methods

Selection of the Subjects

Twenty-two active, healthy footballers, with an age range of 18-32, free of obvious health problems and medications were enrolled into this study. In the study, the necessary explanations were provided to the subjects and their consents were obtained.

Method of the Study

The subjects carried out the test after a 20-min warm-up, 15 min of which was dedicated to general and 5 min to specific warm-up. The test comprised 10 sprints of 20 m. The subjects started the sprint without a start order, but when they felt ready and completed it in maximum speed. Blood measures were taken twice, before and immediately after the sprint.

Instruments

Height and Weight Measure

The heights of the subjects were measured without shoes, with a stadiometer (Holtain, UK) in cm; and they were weighed in shorts with an electronic scale (Seca, Germany) in kg.

Speed Measure

The speed was measured in a gym on a smooth and synthetic floor. The time between the start and finish points were measured with a 0.01 s sensitive photocell system (New Test 2000, Finland). Cones were used in order to specify the sprint field.

Blood Measures

Blood samples were drown from antecubital vein using the heparinised tubes. The samples were taken before and immediately after the loading. The elements were precipitated by centrifuge for 5 min at 3500 rpm; the plasma part at the top was taken into Eppendorf tubes and kept at -80 °C until analysis.

Nitrite determination

Nitrite was measured using the Griess reaction (10). Briefly, plasma samples were diluted 4-fold with distilled water and deproteinized by adding l/20th volume of zinc sulfate (300 g/l) to give a final

concentration of 15 g/l. After centrifugation at 10,000×g for 5 min at room temperature (or 1000×g for 15 min), 100 µl of supernate was applied to a microtiter plate well, followed by 100/µl of Griess reagent (1 g/l sulfanilamide, 25 g/l phosphoric acid, and 0.1 g/l N-1-naphthylethylenediamine). After 10 min of colour development at room temperature, the absorbance was measured on a microplate reader (Tiertek Multiskan MCC/340; Flow Lab, McLean, VA) at a wavelength of 540 nm. Each sample was assayed in duplicate wells. Background values were obtained by treating samples as described but by using 25 g/l phosphoric acid instead of complete Griesa reagent. Calibration curves were genereated with sodium nitrite and potassium nitrate in distilled water (linear range 0-100 µmol/l). The detection limit of the assay is ~1.5 µmol/l in distilled water. There was no difference between the spectrum of authentic nitrite in distilled water and the spectra of negative plasma samples supplemented with exogenous nitrite or the spectra of positive plasma samples. In all these specimens, maximal absorbance occurred at 540 nm (data not shown). The molar absorptivity of the coloured product was 39,500 l mol^{-1} cm^{-1}

Nitrate determination

Nitrate was measured as nitrite after enzymatic conversion by nitrate reductase (EC 1.6.6.2) as described by Schmidt et al. (11). Briefly, 100 µl of plasma was diluted 4-fold with distilled water. NADPH, FAD, and nitrate reductase from *Aspergillus* spp. (Boehringer Mannheim, Mannheim, Germany) were added to yield final concentrations of 50 µmol/, 5 µmol/l, and 200 U/l, respectively.

Samples were further incubated for 5 min at 37 °C to oxidize NADPH [which interferes with the assay] (11), deproteinized, and assayed with Griess reagent as described above. Values obtained by this procedure represent the sum of nitrite and nitrate. Nitrate concentrations were obtained by subtracting nitrite concentrations from the total nitrate + nitrite concentrations.

Statistical Analysis

SPSS 16.0 statistics program was used in analysing the data obtained. The arithmetic mean and standard deviation of all the variants of the footballers included in the study were calculated. Paired samples t-test was used to compare the NO values taken before and immediately after the speed exercise. The difference was assumed significant if the p value was below 0.05.

Results

The demographic results of the group were summarized in Tables 1 and 2. NO values were found to be 21.45 ± 2.08 immediately after the exercise whereas they were 25.59 ± 1.59 before the exercise. This decrease immediately after the exercise, in comparison with before, was not a statistically significant (P = 0.052, Figure 1).

Table 1. Age (years), height (cm) and weight (kg) of footballers.

	Arithmetic Mean	Standard Deviation	Minimum	Maximum
Age (years)	24.21	3.41	18.00	32.00
Height (cm)	173.2	7.04	162	185
Weight (kg)	67.85	5.17	62.32	77.67

Table 2. Serum NO (U/Ml) levels of footballers before speed exercise and immediately after speed exercise.

		Arithmetic Mean	Standard Deviation	t	p
SERUM	Before Exercise	25.59	1.59	1.980	.052
NO U/ml	Immediately After Exercise	21.45	2.08		

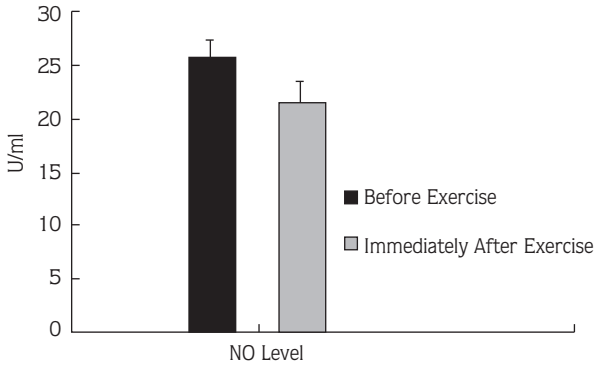

Figure 1. Average serum NO (U/ml) Levels of footballers before and immediately after speed exercise.

Discussion

Recent studies show that exercise has different effects on NO level. Presumed reasons for those differences are the type, duration, and degree of exercise. Particularly acute and heavy exercises can trigger oxidative damage (12). The proportions of oxidants and antioxidants to emerge during the exercise vary according to the degree of exercise. While damaging oxidant system is more activated during heavy and rigorous exercises, regular and short-term sport activities that are not maximal activate the antioxidant systems better (13).

There are various basic sources of oxidant products, such as NO products, oxygen radicals formed through purine metabolism, and prostaglandin. NO products, like peroxinitrites, can contribute to oxidative damage (14). NO, thus, can contribute to cell damage in many cells.

Xia and colleagues studied the effect of different degrees of exercise on NO level and they showed that there was no significant change in NO in low and mild level exercises, whereas there was an increase in NO concentration in high level exercises (15). In contrast to that study, we noted that there was a 5 U/ml decrease in NO levels after the exercise in comparison with those before the exercise. This can be attributed to a set of factors. One of them is the contribution of NO to antioxidant defence in the fight with free radicals occurring in acute exercises of maximal level (16).

Yamamato et al. state that there is no meaningful difference between the plasma and serum NO levels before and immediately after exercise for aerobic type physical exercise (17).

In another study, Keçetepen and Dursun found a decrease in NO values immediately after exercise in a study where sportsmen, who do sports regularly, perform exercise for 2 min consecutively at 30, 40, 50 km/h against 100 W power, and then at maximum speed, using a bicycle ergometer (18).

Radak et al. also state that muscular damage can occur in connection with the increase in NO_X production in skeletal muscles during eccentric contraction (19). Cuzzolin et al. state in the study they carried out on 6 active and 6 sedentary subjects that acute exercise can lead to NO_X formation (20).

In another study, a decrease was observed in nitric oxide synthesis inhibition and active blood flow after the dynamic knee-extensor exercise. In the same study, NO inhibition was greater in heavy exercise when compared with low level exercise (21).

Another study concludes that NO is not essential for the good performance of skeleton muscles in healthy humans (22).

Jungestern et al. compared long-distance runners and students who do not exercise regularly and came up with a meaningful difference in NO levels of long distance runners after exercise in comparison with those before exercise. Furthermore, there was a meaningful increase in NO levels of students who do not exercise regularly after exercise in comparison with before (23).

In our study, the NO levels may have been found to be low for that moment since the blood samples were taken immediately after maximal loading. As a result of the increase in radicals during heavy exercise, it is possible for some of the NO to be directed to radicals. However, different results could have been obtained if blood samples had been taken at regular intervals after exercise.

The fact that the exercise was short-term yet with maximal load and the negative developments that occurred within the body may have highlighted the oxidative damage. The NO defence used against this damage may have decreased the values.

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