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Effects of acute aerobic exercise on NADPH oxidase levels in trained male subjects

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ABSTRACT

Exercise induced muscle activity causes increase in oxidative stress and free radical levels. Cardiac and skeletal muscle activity results in elevated NADPH oxidase levels in body. In the present study, we intended to evaluate NADPH oxidase levels in response to the aerobic exercise performed in the morning and at night in trained male subjects.

Total of 10 healthy trained subjects (age: 19.3±0.7 years and height: 171.8±2.2cm) performed 2 aerobic running exercise in the morning and at night on different days. Venous blood samples were taken at onset and after exercise. NADPH oxidase levels were measured using ELISA method.

NADPH oxidase levels were found as 355.17±48.65 nmol/L at basal and increased to 374.42±47.41 nmol/L ($p<0.0001$) in morning exercise. In addition, it was 353.42±56.07 nmol/L at basal and increased to 368.68±55.12 nmol/L after exercise at night ($p<0.0001$). During exercise, observation of increased percent of NADPH oxidase levels was 6.20±1.8 % in morning and 6.28±1.3 % in night exercise, and no statistical significance was found ($p=0.2$).

As a result, acute aerobic exercise may cause an increase in NADPH oxidase levels in trained subjects but it was not dependent with the time of exercise.

Key Words: Exercise, free radicals, NADPH oxidase, aerobic fitness

Introduction

Regular exercise or physical activity is used commonly as a complementary treatment method against cardiovascular, respiratory, metabolic and musculoskeletal system diseases (1, 2). It is shown in research studies that individuals with augmented aerobic fitness due to regular exercise have lower incidence of diseases compared to sedentary individuals or people with low fitness level (1).

Although exercise activities pose positive effects on body and organ systems, increased oxidative stress and free radical levels produces some harmful side effects (3-5). Under normal conditions free radicals are produced during metabolic activities and have important roles in apoptosis and activities of antioxidant systems (6). However, production of excessive free radicals cause important damages such as oxidation of proteins, nucleic acids and lipids, deterioration of enzyme activities and damage of cell membrane (7, 8).

Nicotine amid adenine dinucleotide phosphate (NADPH) oxidase act as an important potential superoxide source to mammalian cells via transferring an electron to molecular oxygen (9, 10). NADPH is determined in sarcoplasmic reticulum, transverse tubules and sarcolemma in muscle fibers and causes an increase in reactive oxygen species (ROS) in skeletal muscles during exercise activities (11). NADPH oxidase is the major ROS source in cardiac tissue (12-15) and has important role in ischemia related situations (16). Augmented NADPH activity is a general indicator of pathological states such as cardiac hypertrophy (17) and was shown to increase following physiological stimulus during exercise (18). On the other hand regular exercise was shown to attenuate body NADPH levels (19).

Aim of this study is to investigate blood NADPH oxidase level in individuals with high aerobic capacity during exercise performed in the morning and at night.

Materials and Methods

In this study 10 trained male subjects were involved. Physical properties of volunteers are given in Table 1. Prior to study an ethical permission was obtained from Firat University Local Ethic Committee. All contributors to the study were volunteered into the study after signing

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Table 1. Physical characteristic of the subjects, body mass index (BMI), fat free mass (FFM) and fat mass (FM). Data is presented as mean±S.E.M.

Age (year)	19.3±0.7
Height (cm)	171.8± 2.2
Weight (kg)	59.9±2.4
BMI (kg/m2)	20.2±0.6
Total Body Water (kg)	38.8±1.2
FFM (kg)	53.11±1.7
FM (kg)	6.8±0.7

"Informed Consent Form". Volunteers were informed in detail about all possible conditions that may occur during exercise. Volunteers were also mentioned not to do heavy exercises during 24 hours of time period prior to aerobic tests.

Body composition of volunteers was assessed in the morning with bioelectric analysis apparatus (BIA) from feet to feet (Tanita, Body Composition Analyzer, TBF-300 M). This assessment included body fat ratio, body fat percentage, fat free body mass, total body water content and body mass index (20).

Criteria for inclusion of volunteers into the study: Trained male subjects between 18-25 age range were involved into this study. Volunteers were physically healthy, without any disease (common cold, throat infection, muscle inflammation, etc.), and without any metabolic, respiratory, cardiac and musculo-skeletal disorder, acute and chronic diseases (diabetes, obesity, allergy, myocard failure) that may interfere results. In addition, subjects with alcohol, cigarette and drug use habits were not included into the study. Criteria for inclusion are performing sports activities as licensed amateur or professional sportsmen (at least 3 years of sports background and performing regular exercises 3-4 times a week). Volunteers are mentioned not to take any antioxidant drug or vitamin supplement during study period.

Exercise protocol: Volunteers were subjected to aerobic running exercise in the morning between 08:00-10:00 and at night between 20:00-22:00 hours (performed prior to food intake or 3 hours after light food consumption). It was advised not to make alterations in their food intake and avoiding energy drinks, vitamins, coffee and substances that may alter performance. Between two exercise tests a pause of 3 days was given. Aerobic running exercise period was continued approximately for 30 min. Maximal expected heart rate of volunteers were calculated with Karvonen method (21). Aerobic exercise intensity was arranged to levels which cause 64-67% of maximal heart rate which is set by American College of Sports Medicine (22). For heart rate monitoring a commercial heart rate monitoring watch was used (Polar Heart Watch T31-CODED, China).

Blood collection and experimental procedure: From the volunteers involved in the study 5 cc of blood sample was obtained from antecubital vein just before and after the exercise. Blood was transferred into EDTA containing tubes in 2 minutes of blood sampling. Packed erythrocytes are used in determination of NADPH oxidase level. Obtained blood samples were centrifuged at +4 ^{o}C and 4500 rpm for 5 min. Then serum or plasma was removed. Following removal of serum and plasma as well as thrombocyte buffy coat, packed erythrocytes were obtained. Saline was added in equal amount of packed erythrocyte pellet. It was centrifuged at +4 ^{o}C and 4500 rpm for 5 min. Supernatant was removed and equal amount of saline with pellet was added and this procedure was repeated 3 times. Erythrocyte pellet remaining below was kept at -80 ^{o}C until analysis.

NADPH oxidase level was determined by a commercial kit (YH Biosearch Laboratory, Room 1306, Building 1, Lane 60, Zhongyuan Building, Central PlaINS Road, Yangpu District, Shanghai, China Catalog Number: YHB2156Hu) using enzyme linked-immunosorbent assay (ELISA) method. The intra- and inter-assay coefficients of variation and sensitivity for NADPH oxidase were lower than 10 % and 12 %, respectively. Analysis range was; 2 nmol/L-600 nmol/L and its sensitivity was; 1.04 nmol/L.

Statistical analysis: SPSS 22 programme was used for statistical analysis in this study. Results were expressed as mean±S.E.M. Data did not show normal distribution according to Kolmogorov-Smirnov Z test. Therefore Wilcoxon test which is a nonparametric test was used for evaluation of basal and post-exercise NADPH oxidase levels after morning and night exercises. Statistical significance was accepted as $P<0.05$.

Results

Mean NADPH oxidase level of volunteers during aerobic exercise was 355.17±48.65 nmol/L (mean±SE) for basal value and increased after exercise and elevated to 374.42±47.41 nmol/L value ($p<0.0001$) (Figure 1). Prior to night exercise basal NADPH oxidase level was 353.42±56.07 nmol/L and it was elevated to 368.68±55.12 nmol/L ($p<0.0001$) (Figure 1). No statistically significant difference was found between morning and night basal NADPH

oxidase levels and post exercise NADPH oxidase levels.

Mean percent increase rates observed in NADPH oxidase levels during exercise varied between individuals as 6.20±1.8 % in the morning and as 6.28±1.3 % at night but no statistically significant difference was found (p=0.2) (Figure 2).

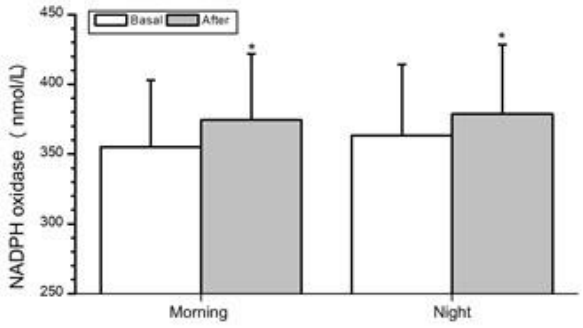

Fig. 1. Mean (± S.E.M.) values of NADPH oxidase in baseline (white column) and at the end of exercise (grey column) in the morning and at night. *represent statistically significant differences between basal and end-exercise values ($p < 0.05$).

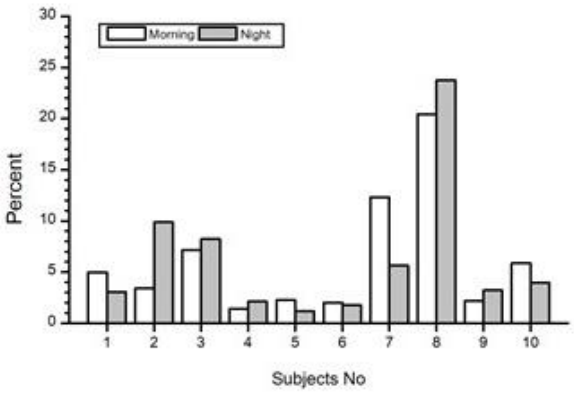

Fig. 2. Percent change of NADPH oxidase levels for each subjects during exercise performed in the morning (white column) and at night (grey column) compared to basal level.

Discussion

This study was conducted to determine any alteration of changes in blood NADPH oxidase levels due to acute aerobic exercise time which was held in the morning and at night time.

Acute aerobic exercise caused significant increases in blood NADPH oxidase levels of all volunteers (Figure 2) which is also reported in existing literature (11, 23, 24). On the other hand no significant difference was observed between basal NADPH oxidase levels and increase rates between morning and night exercises (Figure 2).

NADPH oxidase was reported as one of the most active ROS sources in cardiovascular system (25). Since NADPH oxidase is released from vascular endothelial cells it is an important stress parameter used for assessment of cardiovascular system related risks (15, 26). In addition it was reported that NADPH oxidase can be the major superoxide production site in contracting skeletal muscles (10).

Significant increase of NADPH oxidase level in aerobic exercises performed in the morning and at night time periods of day is an indicator of augmentation of free radical level but no observation of a significant change between each other is an important finding. No change in basal NADPH level suggests a toleration of impacts of stress factors during daytime by well trained volunteers. Another important result is the variation of increase rates among volunteers. Increase in NADPH oxidase level in volunteers in this study may be an indicator for higher stress in cardiovascular and skeletal systems for such individuals (27). Chronic exercise is reported to decrease NADPH oxidase activation and super oxide anion production (28). Physical activity causes increase in oxidative stress and cellular damage but triggers endurance and adaptation via stimulating production of antioxidant enzymes (29, 30). It is suggested that ROS production during exercise triggers adaptive signals via heart mitochondria and/or NADPH oxidase. ROS production with NADPH oxidase is also related with pathological stress in heart. However, NADPH oxidase activation is also mentioned to cause progression of cardio-protective phenotypes (31).

It was shown that chronic exercise provides adaptations which diminish exercise induced oxidative stress (23, 32). Exercise training causes decrease in NADPH oxidase level and poses protective effect against atherosclerotic heart disease risk (33). Excessive NADPH oxidase activity causes endothelial dysfunction. On the contrary, regular aerobic exercise was reported to decrease gp91 phox and Nox4 including NADPH oxidase sub units and p22 phox mRNA level. Another mechanism in this context is the development of adaptation to systemic oxidative stress level by exercise (34).

As a result, NADPH oxidase which is a marker of oxidative stress condition due to active work of skeletal and heart muscle increases with acute

exercise. On the other hand observation of no significant difference between basal level and exercise in morning and night periods may arise from protective effect of trained condition of sportsmen. Repetition of this study also in sedentary individuals or individuals with low fitness condition may provide important results in this subject.

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