

EFFECTS OF HIGH SPEED RUNNING ON OXIDATIVE STRESS, MUSCLE AND CARDIAC INJURY PARAMETERS

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Abstract

Introduction: Anaerobic exercise may have diverse pathophysiological effects on various organs due to free radical formation.

Objective: To assess the effects of sixty meter speed running that was performed for once, on plasma parameters of oxidative stress, skeletal muscle and cardiac damage.

Methods: Twenty-two healthy students, aged 18 to 23 years, were included in this study. The subjects were applied a sixty meter speed running test on a dust running track. For biochemical measurements, fasting blood samples were obtained from the subjects both before and after the exercise.

Results: Significant increases in lactate dehydrogenase (LDH), aspartate aminotransferase (AST), creatine kinase (CK), CK-MB and CK-MB mass were observed post-exercise ($P < 0.001$), while cardiac troponin T (cTnT) and myoglobin (Mb) levels were not affected ($P > 0.05$). Plasma malondialdehyde (MDA) levels significantly increased ($P < 0.001$) after the exercise but this was not accompanied by changes in erythrocyte superoxide dismutase (SOD) activity ($P > 0.05$).

Conclusions: The results show that when anaerobically trained men run a comparatively short distance sufficient to result in some degree of muscle damage and in plasma indices of lipid peroxidation, but which is insufficient to cause significant alterations in erythrocyte antioxidant status and markers of cardiac markers do occur.

Key words: exercise, lipid peroxidation, superoxide dismutase, creatine kinase, cardiac troponin T, myoglobin

Introduction

The beneficial effects of regular, non-exhaustive physical exercise have been known for a long time. Regular exercise and exercise training are important in both the primary and secondary prevention of coronary heart disease [1]. However, exhaustion and lack of regular exercise reduce the beneficial effects of exercise. Physical exercise under circumstances such as unaccustomed intensity or duration increases the production of free radicals and leads to oxidative stress [2,3]. Exhaustive exercise, such as endurance exercise, is associated with accelerated oxygen radical formation that results in oxidative stress, which can induce adverse effects on health and well-being [1,4]. Levels of exercise-induced reactive oxygen species (ROS) have been shown to be intensity dependent; that is, lower intensity exercise may not be sufficient to cause a significant oxidative stress [5]. As ROS can serve as cell messengers or modify oxidation-reduction status, they play an important role in cellular signaling. Antioxidant defense system includes both endogenous and exogenous antioxidants. Superoxide dismutase (SOD), one of the most important endogenous antioxidant enzymes, can be activated by an acute bout of exercise at sufficient intensity [5,6]. This can be considered as a defensive mechanism of the cell un-

der oxidative stress. Trained athletes who were given antioxidant supplements showed evidence of reduced oxidative stress; however, it is not yet clear whether the antioxidant defense system of the body is sufficient to neutralize the increase in exercise-induced ROS [6,7]. Exercise-induced ROS is known to be involved in cell and tissue injury, indicating oxidative stress. Lipid peroxidation (LP), polyunsaturated fatty acid oxidation in biological membranes, is the best indicator of oxidative damage, and leads to defects in cell membranes. Malondialdehyde (MDA) is one of the end-products of LP. There are conflicting reports about the effects of oxidative stress on plasma lipid peroxidation levels after acute exercise [8].

It is well known that resistance exercise can damage muscle tissue. Creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) are widely used in the diagnosis of skeletal muscle diseases in clinical practice, as markers of cellular necrosis and tissue damage in skeletal muscles [9]. A number of biomarker assays including CK isoenzyme MB mass (CK-MB mass) and cTnT, which are considered as gold standard tests for cardiomyocyte insult, are commonly used to aid the diagnosis of acute myocardial infarction in emergency cases of suspected heart attack [9]. Exercise-induced muscle damage leads to

an acute-phase inflammatory response characterized by infiltration of the phagocytes into the damaged muscle and production of free radicals. Although maximum speed running involves intense eccentric muscle actions that can cause muscle and cardiac damage, oxidative stress responses after speed running are currently unknown. Training can have positive or negative effects on oxidative stress depending on training load, training specificity, and the basal level of training. It has been demonstrated that there is increased oxidative stress during short-term anaerobic exercise. Exercise-induced muscle damage has been widely reported following different types of eccentric exercise in humans and may lead to transient rise in serum concentration of muscle proteins such as CK and Mb [10,11]. It results in a temporary loss of exercise capacity of the muscle for force production and causes increased muscle soreness [10]. To date, little information is available concerning maximum speed running, applied for once, that utilizes predominantly concentric contractions, and oxidative stress and associated muscle and cardiac damage. The purpose of this study, was to evaluate the effects of sixty-meter maximum speed running, applied once, on the levels of LDH, AST, CK, CK-MB, CK-MB mass, Mb, cTnT, MDA (LP end-product) and SOD activity (antioxidant) in twenty-two healthy male students.

Methods

Participants

Twenty-two healthy male students, aged 18 to 23 (21.64 ± 2.01) years, who were attending the High School of Physical Education and Sports were included in this study. Sixty meter speed running test was carried out only for once on a dust running track. The subjects were physically active but had not been involved in regular high athletic training such as endurance or sprint training. The subjects were recruited based on their weekly activity patterns over the preceding year. Before selection, they completed a questionnaire regarding their diet, medication, and smoking habits. None of the subjects had major medical illness, tobacco use within 6 months prior to the study and none were taking antioxidant compounds, including vitamins and medications (anti-inflammatory agents). All refrained from ethanol-containing beverages for 1 week prior to exercise testing and from physical exercise for at least 2 days before the test. The investigation conformed to the principles outlined in the Helsinki Declaration. Written informed consent was obtained from all participants prior to study entry. The local ethics committee approved the study protocol.

Sample preparation

For biochemical measurements, fasting blood samples were obtained from the subjects both before and

after the exercise. Venous blood samples were drawn into tubes with and without anticoagulant (K_2EDTA) after overnight fasting. The serum was separated from the cells by centrifugation at $1000 \times g$ for 10 minutes at $4^\circ C$ and the serum samples were stored at $-80^\circ C$ until analysis, as described below. The second portion of the blood sample was centrifuged at $1000 \times g$ at $4^\circ C$ for 10 minutes, and the plasma was separated. The buffy coat was removed and the RBCs were washed three times in chilled physiological saline solution, and then hemolyzed in distilled water (1:4, v/v), vortexed, and stored at $-80^\circ C$ until use.

Biochemical assays

The cardiac markers were measured by electrochemiluminescence method (Elecsys 2010, Roche), and the other analytes by automated photometric methods using commercial kits (Modular, Roche).

Hematological parameters

Peripheral blood samples collected in tubes containing K_2EDTA as anticoagulant were analyzed for all routine full blood count parameters, including platelet counts (Sysmex XT-2000i, Tokyo, Japan).

Determination of Erythrocyte SOD activity

Erythrocyte SOD activity was measured using the commercial Ransod reagent (Randox Laboratories, Crumlin, UK) and is based on the method developed by McCord and Fridovich, coupling O_2^- generators [xanthine and xanthine oxidase (XOD)] with an O_2^- detector [2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride; I.N.T.]. The activity is defined as the amount of SOD that inhibits the rate of formazan dye formation by 50%. The activity of SOD in units per liter divided by hemoglobin (Hb) in grams per liter gives the activity of SOD in units per gram of Hb. The measurements were performed on a Hitachi 902 automated analyzer (Roche Diagnostics, Hitachi, Tokyo, Japan).

Malondialdehyde analysis

MDA is analyzed by a method based on the reaction with thiobarbituric acid under high temperature; the product forms a pink color that can be measured spectrophotometrically. The procedure consists of the addition of 1.5 mL of 20% acetic acid, 1.5 mL of 0.8% thiobarbituric acid, 200 μL of 8.1% sodium dodecyl sulphate, and 700 μL of distilled water to a 100 μL sample. The reaction mixture was heated in boiling water for 60 minutes. After cooling, 1 mL of distilled water and 5 mL of butanol/pyridine (14/1: v/v) were added into the samples. Then, the samples were centrifuged at $900 \times g$ for 10 minutes. Absorbance of the supernatant was measured at 532 nm. The amount of thiobarbituric acid reactive substances was calculated

as MDA equivalents using 1,1,3,3-tetramethoxypropane as standard.

Statistical analysis

The results are presented in absolute values as mean $\pm$ standard deviation. The Kolmogorov-Smirnov test was used to test the normality of the distribution. Statistical comparisons between pre- and post-speed run values were performed by a Student paired *t*-test. Spearman test was used to analyze the relationship between in post-exercise biochemical parameters. The significance level was set at 5%.

Results

The anthropometric and physiological characteristics of the subjects are given in Table 1. As documented in Table 2, the hematological parameters showed drastic changes. Complete blood count (CBC) parameters after exercise than before exercise showed a significant increase ($P < 0.001$), except mean corpuscular hemo-

globin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet (Plt), and eosinophil counts. There was a slight but significant decrease in MCHC ($P < 0.05$).

Heart rate and serum AST, LDH, CK, CK-MB, CK-MB mass, Mb, cTnT, MDA levels and erythrocyte SOD activities of the subjects at pre- and post-run are shown in Table 3. Exercise induced a significant increase in the levels of AST, LDH, CK, CK-MB, CK-MB mass and heart rate ($P < 0.001$). On the other hand, Mb and cTnT levels were not significantly changed ($P > 0.05$). After the exercise, plasma levels of MDA significantly increased ($P < 0.001$) while erythrocyte SOD activities were not significantly affected ($P > 0.05$) compared to pre-exercise levels.

After exercise possible correlation between biochemical and oxidative stress parameters were evaluated. In fact, significant correlations were only found between biochemical variables. No significant correlations were found between any oxidative stress

Table 1. *Anthropometric and physiological characteristics of the subjects*

Variables	Mean $\pm$ Standard Deviation
Age (years)	21.41 $\pm$ 1.47
Height (m)	1.74 $\pm$ 0.05
Body Weight (kg)	69.45 $\pm$ 4.53
Body mass index (kg/m ²)	22.9 $\pm$ 1.79
% Body fat	12.68 $\pm$ 2.63
Systolic blood pressure (mm-Hg)	114.7 $\pm$ 8.60
Diastolic blood pressure (mm-Hg)	66.0 $\pm$ 6.84
Anaerobic power (kg·m/s)	113 $\pm$ 8.6
Mean time for run (s)	8.3 $\pm$ 0.36

Table 2. *The comparison of the pre- and post- exercise complete blood count parameters of the subjects*

	Pre-exercise	Post-exercise	Significance
White blood cell ($\times 10^3/\mu\text{L}$)	7.57 $\pm$ 0.35	10.30 $\pm$ 0.43	**
Eosinophil ($\times 10^3/\mu\text{L}$)	0.273 $\pm$ 0.04	0.282 $\pm$ 0.05	Ns
Basophil ($\times 10^3/\mu\text{L}$)	0.0 $\pm$ 0.05	0.03 $\pm$ 0.01	*
Lymphocyte ($\times 10^3/\mu\text{L}$)	2.54 $\pm$ 0.12	3.54 $\pm$ 0.14	**
Monocyte ($\times 10^3/\mu\text{L}$)	0.41 $\pm$ 0.03	0.74 $\pm$ 0.05	**
Neutrophil ($\times 10^3/\mu\text{L}$)	4.33 $\pm$ 0.28	5.71 $\pm$ 0.39	**
Red blood cell ($\times 10^6/\mu\text{L}$)	5.41 $\pm$ 0.06	5.62 $\pm$ 0.06	**
Hemoglobin (g/dL)	16.15 $\pm$ 0.16	16.82 $\pm$ 0.15	**
Hematocrit (%)	46.13 $\pm$ 0.45	48.63 $\pm$ 0.47	**
Mean Corpuscular Volume (fl)	85.27 $\pm$ 2.47	86.51 $\pm$ 2.38	**
Mean Corpuscular Hemoglobin (pg)	29.86 $\pm$ 0.90	29.95 $\pm$ 1.03	Ns
Mean Corpuscular Hemoglobin Concentration (g/dL)	35.01 $\pm$ 0.43	34.60 $\pm$ 0.52	*
Platelet ($\times 10^3/\mu\text{L}$)	232.09 $\pm$ 12.61	235.09 $\pm$ 13.81	Ns

Ns: Not significant

** $P < 0.001$ * $P < 0.05$

Table 3. *Biochemical markers, oxidative stress parameters and heart rate of the subjects at pre-exercise and post-exercise period*

	Pre-exercise	Post-exercise	Significance
Heart rate (bpm)	62±7.3	135±11.9	**
Aspartate aminotransferase (U/L)	21.36±1.47	24.86±1.74	**
Lactate dehydrogenase (U/L)	297.55±12.37	353.50±15.67	**
Creatine kinase (U/L)	285.14±60.76	312.91±65.73	**
Creatine kinase-MB (U/L)	16.09±1.42	18.64±1.42	**
Creatine kinase-MB mass (ng/mL)	2.50±0.41	3.01±0.50	**
Myoglobin (ng/mL)	42.12±2.54	42.57±2.57	Ns
Cardiac troponin T (ng/mL)	<0.1	<0.1	Ns
Malondialdehyde (nmol/mL)	6.06±0.40	6.87±0.42	**
Superoxide dismutase (U/gHb)	2183.29±43.17	2153.14±45.23	Ns

** $P < 0.001$

Table 4. *The significant correlations between the biochemical parameters*

Parameters	Correlation coefficient (r)
AST: CK	0.865**
AST: CK-MB	0.748**
AST: CK-MB mass	0.853**
AST: LDH	0.806**
AST: Mb	0.807**
LDH: CK-MB	0.569**
LDH: CK	0.780**
LDH: CK-MB mass	0.743**
LDH: Hct	0.324*
LDH: Mb	0.716**
LDH: WBC	0.435*
Mb: CK	0.840**
Mb: CK-MB	0.700**
Mb: CK-MB mass	0.770**

AST: Aspartate aminotransferase; CK: Creatine kinase; LDH: Lactate dehydrogenase; Mb: Myoglobin; Hct: Hematocrit; WBC: White blood cell;

** $P < 0.001$

* $P < 0.05$

and damage markers analyzed. AST had a high correlation with CK, CK-MB, CK-MB mass, LDH and Mb ($P < 0.001$). LDH was correlated to CK, CK-MB, CK-MB mass, Mb ($P < 0.001$) and hematocrit (Hct), white blood cell (WBC) count ($P < 0.05$). At the same time Mb had a high correlation with CK, CK-MB and CK-MB mass ($P < 0.001$).

Discussion

Speed running test leads to several acute physiological changes such as increased cardiac output and blood flow, increased catecholamine release, high eccentric demand and mobilization of blood leukocytes and it relies on aerobic metabolism. Many studies have reported that physical exercise increases ROS production; thereby induce oxidative stress [12-14]. The majority of these studies utilized aerobic exercise

to study the levels of ROS. It has been suggested that elevated oxygen consumption leads to the generation of increased levels of ROS [15,16].

The aim in this study was to investigate lipid peroxidation, antioxidant status, muscle and cardiac damage in a period of anaerobic running to exhaustion, in twenty-two healthy male students. The extent of oxidative damage during physical exercise is determined not only by the level of ROS generation but also by antioxidant defense capacity. It has been reported that an acute bout of exercise increase SOD activity in a number of tissues and red blood cells [17,18]. Schneider et al. found a significant increase in SOD activity after low- and moderate- intensity exercise and a non-significant increase after high intensity exercise in untrained subjects [19]. However, Wang et al reported no significant change in plasma SOD activity after mild,

moderate, and heavy exercise [20]. Balci et al. [6] found increased SOD activity along with MDA during high-intensity walking exercise, but no difference with the other exercise tests. On the other hand; Powers et al. [21] suggested that chronic endurance exercise training did not increase SOD activity in the muscle. In this study, we observed that the erythrocyte SOD activities were not significantly different between pre- and post-anaerobic exercise ($P > 0.05$), whereas plasma MDA levels were significantly higher in the post-exercise period ($P < 0.001$). The increased oxidative damage induced by speed running can additionally be demonstrated by the accumulation of lipid peroxidation by products, measured as plasma level of MDA, post-exercise. Recent reports have suggested that mild and regular exercise can increase the antioxidant capacity. From this point of view, the cells are protected from the injury caused by free radical production because antioxidant levels increase in those who exercise regularly. The general opinion is that physical activity plays a beneficial role in the prevention of disease [1,21-23]. Considering that most ROS are able to diffuse through the RBC membrane, and that RBCs have limited repair mechanisms, increased oxidative stress could alter the chemical and physical properties of erythrocyte cell membranes by modifying the composition, packaging, and distribution of their lipids [24]. In this present study, post-exercise RBC count was significantly increased compared to that of pre-exercise values ($P < 0.001$), with higher Hb concentration per cell, as shown by the significantly higher values of Hct and MCV ($P < 0.001$), while MCHC was decreased significantly ($P < 0.05$). In accordance with our results, Hu et al showed that resistance training significantly increased RBC count and Hct, and decreased the MCHC in physically inactive men [18].

RBCs are sensitive to oxidative damage as they are continuously exposed to oxygen and have high concentrations of polyunsaturated fatty acids. Oxidative damage may lead to hypoxia in the working muscle during single episodes of exercise and to an increased rate of RBC destruction with long term exercise [25,26]. Provided that destruction of RBCs does not exceed the rate of production, there should be no harmful effect on athletic performance. On the other hand, an increased rate of RBC turnover may be advantageous as young RBCs are more efficient in oxygen transport [27]. We found a significantly increased WBC count in the post-run blood ($P < 0.001$), the count of neutrophils, lymphocytes and monocytes were also significantly increased ($P < 0.001$). In consideration of the exercise-induced neutrophilia, the capacity of the runner's blood for oxygen radical generation increased during speed running. Leukocytosis, with neutrophilia, is well documented after exhaustive exercise such as soccer training [28]. Hessel et al [29] reported that activated neutrophils, an important oxy-

gen radical source, could be mobilized during exhaustive exercise. Neutrophils and macrophages contribute to the degradation of damaged muscle tissue by releasing reactive oxygen and nitrogen species, and may also produce pro-inflammatory cytokines. Increased oxygen radical generation of activated neutrophils had been also demonstrated after moderate-intensity running [30]. In agreement with other studies [31,32], we clearly showed that leukocytosis was associated with neutrophilia, which may be attributed to the mobilization of cells from the marginal pool by hemodynamic redistribution and augmentation that resulted from exercise-associated metabolic conditions, such as increased cortisol and catecholamine secretion [33].

The release of myocardial injury biomarkers is common in adults after repeated prolonged exercise bouts [34]. However, the pre- and post-workout values of cardiac biomarkers after high-intensity speed running, as applied once, in young runners, is unclear. In the present study, we found that serum CK, CK-MB, CK-MB mass, AST and LDH increased significantly in all subjects after sixty-meter speed running ($P < 0.001$). The high activity of CK, LDH and AST enzymes in the blood is due to the changes in skeletal muscle membrane permeability as well as to the disruption and death of the cells, as a response to exercise [35]. Nie et al. [9] detected 1.8-, 1.5 and 1.4 fold increases in CK, LDH and AST levels, respectively after a 21 km run in adolescent runners. Significantly higher post-exercise levels of plasma CK was associated with muscle damage, and routine biochemical evaluation of plasma CK levels in the diagnosis of muscle disease has been proposed [6].

In the normal skeletal muscle, CK-MB levels are low but in endurance training CK-MB accumulation in skeletal muscle may reach myocardial levels, which should be interpreted as an indicator of skeletal muscle injury [36]. CK-MB mass differs from conventional CK-MB measurement in that the latter is an indirect measurement of the enzyme activity, while CK-MB mass is a direct measurement of enzyme concentration.

In our study, serum Mb and cTnT concentrations were not affected by speed race in all subjects ($P > 0.05$), and these results suggest that the rate of efflux from tissue into blood may be attenuated by training.

It has been shown that rapidly release of Mb due to muscle damage in intensive exercise, such as a marathon, is accompanied by injury to the skeletal muscle fibers, which results in increases in plasma concentrations of intramuscular enzymes and Mb [36]. The increase seems to be related to the type and intensity of the exercise, and to the previous activity level of the subjects [36,37]. It has been shown that plasma CK levels were correlated positively with Mb concentration, and which is very encouraging as the molecular

mass of Mb (17 kDa) is much lower than that of CK (82 kDa), and generally Mb leaks easily from damaged muscle and disappears sooner from circulation due to renal excretion, however, Carmeli et al. [38] detected that both molecules respond similarly to endurance exercise training.

In this study, we also found that serum AST levels were significantly correlated with CK, CK-MB, CK-MB mass, LDH and Mb concentrations ($P < 0.001$). There was a significant positive correlation between LDH and CK, CK-MB, CK-MB mass, Mb ($P < 0.001$) Hct and WBC levels ($P < 0.05$). Additionally, the concentration of Mb was correlated significantly with CK, CK-MB and CK-MB mass levels ($P < 0.001$). One of the limitations of the present study is that we examined only two markers of oxidative stress; it is possible that other markers of oxidative stress may provide additional information related to exhaustive exercise-induced oxidative stress. Additionally, the study included only moderately active subjects who participated in lessons in the Department of Physical Education and Sports, but not highly physically trained individuals. Therefore, the responses observed may not be representative of either very physically fit or sedentary individuals.

As a result, short duration exhaustive exercise resulted in significant increases in the levels of LDH, CK, and AST, as markers of muscle damage, although it did not change Mb levels. Among myocardial damage markers, the serum CK-MB and CK-MB mass levels were significantly increased while serum cTnT levels were not changed after exhaustive exercise compared with the baseline value. Additionally, short duration, high intensity, exhaustive running exercise caused significant lipid peroxidation whereas it did not significantly change erythrocyte SOD activity.

Declaration of interest

The author(s) declare that they have no conflict of interest.

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