Cryobiology 74 (2017) 126–131

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

The oxidant-antioxidant equilibrium, activities of selected lysosomal enzymes and activity of acute phase protein in peripheral blood of 18-year-old football players after aerobic cycle ergometer test combined with ice-water immersion or recovery at room temperature

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ARTICLE INFO

Article history:
Received 29 June 2016
Received in revised form
21 October 2016
Accepted 17 November 2016
Available online 18 November 2016

Keywords:
Antioxidant enzymes
Lysosomal enzymes
Physical effort
Reactive oxygen species
Low temperature
α1-Antitrypsin

ABSTRACT

The goal of the study was to evaluate the effect of an aerobic exercise bout followed by ice-water immersion or recovery at room temperature on the redox state, activities of selected lysosomal enzymes and activity of α1-antitrypsin (AAT) in the blood of healthy sportsmen. Eleven amateur football players aged 18 were randomly assigned to two similar 30-min aerobic cycle ergometer tests followed by a recovery at room temperature (20 °C; Experiment 1) or ice-water immersion (3 °C, 5 min; Experiment 2). Peripheral blood was collected three times during both study experiments: before (baseline), as well as 20 and 40 min after the recovery or immersion. The concentrations of thiobarbituric acid reactive substances in blood plasma (plTBARS) and erythrocytes (erTBARS) were measured. The erythrocytic activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were also determined. In the blood serum, the activities of acid phosphatase (AcP), arylsulphatase (ASA), cathepsin D (CTS D) and AAT were evaluated. The activities of AcP, ASA, CTS D and AAT changed similarly during both experiments. The GPx activity decreased 40 min after the exercise/recovery compared to the baseline activity and was lower than 40 min after the exercise/immersion. The exercise followed by the recovery or immersion had no significant effect on the serum lysosomal and AAT activities in the studied men. The exercise/recovery reduced the hydrogen peroxide concentration in the men's erythrocytes, however the exercise/immersion demonstrated the opposite effect.

1. Introduction

Reactive oxygen species (ROS) are mostly free radicals—very reactive substances, usually oxidants. Primarily, they are products of incomplete reduction of the oxygen molecule in the mitochondria. O_2 can be reduced by one electron in the electron transport chain during the process of "electron leakage". The process leads to generating O_2^{-} that reacts with superoxide dismutase (SOD). The product of the reaction is H_2O_2 [44]. Hydrogen peroxide has a relatively low reactivity and, moreover, is electrically neutral. This enables diffusion of hydrogen over long distances, which becomes the source of very reactive hydroxyl radical in other tissues in the presence of free transient metals or the source of toxic peroxynitrite in the presence of nitric oxide [38,44]. H_2O_2 is scavenged by

Abbreviations: ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; IWI, ice-water immersion; DOMS, delayed-onset muscle soreness; plTBARS, plasma TBARS; erTBARS, erythrocytic TBARS; AcP, acid phosphatase; ASA, arylsulfatase; CTS D, cathepsin D; AAT, α1-antitrypsin; BIA, bioelectrical impedance analysis; IPAQ, international physical activity questionnaire; VO_{2max}, maximum oxygen uptake; PWC, physical working capacity; RT, room temperature; HRmax, maximal heart rate; TBA, thiobarbituric acid; GSH, reduced glutathione; GSSG, oxidized glutathione; NADPH, nicotinamide adenine dinucleotide phosphate; 4-NCS, 4-*p*-nitrocatechol; 4-NC, 4-nitrocatechol.

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http://dx.doi.org/10.1016/j.cryobiol.2016.11.004

glutathione peroxidase (GPx) and catalase (CAT), thus, together with SOD, they are the most important intracellular antioxidants [19,44].

During physical exercise, the metabolism of oxygen is significantly increased. Oxygen uptake may increase even 20 times compared to the uptake at rest, however, the oxygen consumption in muscles may increase even 200 times [34]. Therefore, a significant increase in ROS concentrations resulting from the exercise may lead to many micro injuries of the muscle tissue and thus to local inflammation and muscle pain [10,34]. Exercise-induced oxidant damage may practically include all endogenous compounds: carbohydrates, lipids, proteins or nucleic acids [2]. Thus, the release of thiobarbituric acid reactive substances (TBARS) [40,46] and lysosomal hydrolases [10,47] into the peripheral blood is closely related to the damage of muscle tissue and lipoperoxidation of sarcolemma and membranes of muscle lysosomes. In turn, post-exercise inflammation is associated with increased activity of anti-inflammatory proteins and some acute phase proteins. The example of such proteins is α1-antitrypsin, an inhibitor of serine proteases, which is both an anti-inflammatory and acute phase protein [29]. Moreover, the prevailing member of TBARS is malondialdehyde (MDA), one of the most specific markers of oxidative damage of lipids. Therefore, changes in TBARS concentrations allow determining changes in the level of lipoperoxidation of cell membranes [17].

The increase in ROS levels can also occur in response to external stimuli [38], e.g., low temperature [6]. It has been demonstrated that ice-water immersion (IWI) may induce such reaction [37]. Therefore, according to the literature, it may constitute a treatment that adapts human organism to high concentrations of ROS. Moreover, this type of cryostimulation activates the release of high amounts of noradrenaline and/or adrenaline [22] and cortisol [21]. High levels of these hormones in blood after IWI is probably responsible for the analgesic and anti-inflammatory effect of the immersion in the treatment of rheumatic diseases (fibromyalgia, chronic arthritis) [22,39]. IWI often takes the form of winter swimming in natural water reservoirs (rivers, lakes). In such conditions, water temperature is between 0 °C and 4 °C and air temperature is below or a few degrees above 0 °C, however, the duration of such exposure ranges from tens of seconds to a few minutes [30,37,39]. Cold stimulation including IWI is widely practiced also in sports as a simple and fast procedure that accelerates post-exercise recovery. It is also believed that besides the rapid cooling of the body, IWI reduces exercise-induced muscle pain. However, recent studies indicate also the opposite effect indicating that IWI does not relieve the symptoms of delayed-onset muscle soreness (DOMS) after intense exercise [35].

Therefore, the aim of the study was to examine the erythrocytic activity of SOD, CAT and GPx, as well as the plasma and erythrocytic TBARS concentrations (plTBARS and erTBARS, respectively) in the peripheral blood of 18-year-old trained men after a 30-min aerobic exercise test on a cycle ergometer followed directly by a 5-min IWI or recovery at room temperature. The activities of selected lysosomal hydrolases were also measured in the blood serum of the men: acid phosphatase (AcP), arylsulfatase (ASA), cathepsin D (CTS D). In the serum, the activity of serine proteases inhibitor/acute phase reactant, α1-antitrypsin (AAT), was also determined.

2. Material and methods

2.1. Subjects

The study involved 11 healthy 18-year-old men, amateur footballers. The subjects had never immersed themselves in icy water and did not change their eating habits and physical activity immediately before or during the study (about 1.5 months). The footballers practiced physical exercise regularly 5 times a week and the start of the study occurred 2 days (48 h) after the last training session of the preceding week. The studied group was homogeneous regarding physical parameters (Table 1). Baseline body composition was assessed using a body composition analyser (Tanita BC 418 MA) via bioelectrical impedance analysis (BIA). The level of physical activity of the footballers 7 days before the study was high according International Physical Activity Questionnaire (IPAQ) [24]. Baseline aerobic fitness was average, but in the upper limit of normal, and was expressed by maximum oxygen uptake (VO_{2max}) calculated via an indirect method—the physical working capacity 170 (PWC170) test [18]. In the method, VO_{2max} was determined graphically based on the obtained PWC170 index (work performed with the heart rate of 170 beats per minute and expressed in watts) and a read from Astrand–Ryhming nomogram [1]. The subjects were informed about the purpose of the study and gave their written consent. The study received the approval of the Bioethics Committee at the Collegium Medicum in Bydgoszcz of Nicolaus Copernicus University in Toruń, Poland (KB 657/2012).

2.2. Procedures

In Experiment 1 of the study, the subjects performed a 30-min exercise bout on a cycle ergometer (Monark 828 E) and rested for 10 min in a sitting position at room temperature (RT, 20 °C), drinking still water (200 mL, rehydration) (exercise/recovery combination). In Experiment 2 (three days later), the participants performed a similar 30-min exercise bout but immediately after that they immersed themselves (excluding head) in a pool of icy water (3 °C) for 5 min (IWI). Total duration of the immersion was 10 min as well, including getting dressed and rehydration with water. Moreover, the intensity of the 30-min exercise bout in Experiment 1 reached 79.3% and in Experiment 2 reached 77.2% of maximal heart rate (HRmax). HRmax was calculated according to Tanaka's formula for people who practice endurance disciplines: HRmax = 206−0.7 × age [42]. Both in Experiment 1 and 2 of the study, blood for laboratory assays was taken three times from the cubital vein into plastic tubes with K_2EDTA for plasma and with serum clot activator: before the exercise (baseline), as well as 20 and 40 min after the recovery at RT or after IWI.

2.3. Outcomes

All measurements were based on spectrophotometric methods. plTBARS and erTBARS concentrations were determined (erTBARS) using the Buege and Aust method [9] as modified by Esterbauer and Cheeseman [17]. The method is based on the detection of MDA in the sample using thiobarbituric acid (TBA). The erythrocytic CAT (E.C. 1.11.1.6) activity was measured in erythrocytes using the Beers

Table 1
Characteristics of the studied men. Values are shown as mean ± SD.

Subjects' number	11
Age (years)	18.1 ± 2.0
BM (body mass, kg)	70.2 ± 7.4
BH (body height, cm)	178.8 ± 6.4
BF (body fat, %)	14.4 ± 1.9
TBW (total body water, %)	62.7 ± 1.4
MM (muscle mass, %)	45.5 ± 1.5
BMI (body mass index, kg/m)	21.9 ± 1.8
BS (body surface, m^2)	1.9 ± 0.1
IPAQ (international physical activity questionnaire, MET·min/wk)	4185.2 ± 1669.1
VO_{2max} (maximum oxygen uptake, mL/kg/min)	49.3 ± 4.8