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Oxidation–reduction processes in ice swimmers after ice-cold water bath and aerobic exercise ☆

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ABSTRACT

The effect of an ice-cold water (ICW) bath as a recovery intervention from aerobic exercise on the oxidant–antioxidant balance in healthy ice swimmers was determined. Twenty ice swimmers aged 31.2 ± 6.3 years performed a 30-min cycloergometer exercise test at room temperature (20 °C, RT), followed by recovery at RT or in a pool of ice-cold water (ICW bath, 3 °C, 5 min). Blood for laboratory assays was collected from the basilic vein two times: before the exercise (baseline) and 40 min after the RT or ICW recovery. The concentrations of plasma and erythrocytic thiobarbituric acid reactive substances (plTBARS and erTBARS, respectively), serum concentrations of 8-iso-prostaglandin F2α, 4-hydroxynonenal and malondialdehyde, along with the erythrocytic activities of catalase (CAT) and superoxide dismutase (SOD), as well as the serum level of total antioxidant capacity, were assessed. No statistically significant changes were observed. However, a statistically significant negative linear correlation between the erTBARS concentration and the SOD activity was found 40 min after the combination of exercise/RT recovery ($r = -0.571$, $P < 0.01$). The baseline CAT and SOD activities were also linearly correlated ($r = 0.469$, $P < 0.05$). Both the 5-min ICW bath and the 30-min aerobic exercise have practically no impact on the oxidant–antioxidant balance in healthy ice swimmers.

Introduction

Healthy human organism attempts to maintain equilibrium between oxidants and reducers. The equilibrium is called the oxidant–antioxidant balance. An increase in the generation of reactive oxygen species (ROS) or a decrease in antioxidant activity induces a disturbance of the balance and promotes oxidation [25]. Subsequently, the stationary concentrations of ROS become significantly increased and oxidative stress occurs as the oxidation and subsequent functional impairment of lipids, proteins, and nucleic acids [1]. The cause of oxidative stress may be an increase in the rate of ROS production that results from the deficiency of low molecular weight antioxidants (e.g., glutathione or antioxidant vitamins) or insufficiency of antioxidant enzymes, specifically: superoxide dismutase, catalase and glutathione peroxidase [1,25]. Oxidative stress may be physiological (e.g., as a consequence of physical exercise [25], reperfusion following ischaemia [1], ageing [8]) or pathological, i.e. occurring in the course of most diseases [21]. Physiological oxidative stress also occurs after exposure to low temperatures. Cooling of the human body results in an ischemia/anoxia episode caused by the contraction of skin blood vessels. The subsequent rewarming, as a consequence of the return to physiological conditions, leads to hyperemia/reoxygenation and the conversion of xanthine dehydrogenase into xanthine oxidase in the vessels. Activated xanthine oxidase becomes the source of ROS [26]. Moreover, in rats, hypothermia and recovery from this state is associated with increased lipid peroxidation and decreased activities of the aforementioned antioxidant enzymes [1]. Nevertheless, there have been many reports describing the beneficial effects of the exposure to low temperatures when applied during pre-exercise stimulation [14,27], recovery from exercise [3] or with no exercise involved [13,19]. According to the literature, the regular use of procedures involving cold, whose effects result from the trigger levels of ROS, may induce or modulate extrinsic and intrinsic signaling pathways, ultimately increasing the abilities of

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the antioxidant defense system, exerting an anti-inflammatory effect and limiting inflammation-induced tissue damage caused by physical exercise [3,13,14,19,27]. It should also be noted that most papers on the cold therapy in sports describe the use of whole-body cryotherapy (WBC) [5]. However, the use of ice-cold water (ICW) bathing is more justified primarily due to the considerably lower expense of the method compared to WBC and similar impact on physiological and clinical events [13,19]. Thus, the aim of the study was to determine the effect of a single and merely 5-min ICW bath on the oxidant–antioxidant balance in healthy volunteers who had such baths regularly in the winter season. Therefore, in the study subjects' peripheral blood, the concentrations of plasma and erythrocytic thiobarbituric acid reactive substances (plTBARS and erTBARS, respectively), serum concentrations of 8-iso-prostaglandin F2α (8-iso-PGF2α), 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA), along with the erythrocytic activities of catalase (CAT) and superoxide dismutase (SOD), as well as the serum level of total antioxidant capacity (TAC), were determined. TBARS, 8-iso-PGF2α, 4-HNE and MDA are lipid peroxidation markers [20,22], while the enzymes are the most important antioxidant agents [25]. All these parameters are very sensitive indicators of oxidative stress [13,28]. Moreover, they have been used many times as the markers of the disturbance of the oxidant–antioxidant balance in response to physical exercise and environmental extremes [14–15,26,28].

Material and methods

Participants

The study included 20 male ice swimmers from Olsztyn, Poland, aged 31.2 ± 6.3 years. In the winter season, the men from the investigated group regularly (once a week) had an ICW bath in the Wadąg river in Olsztyn. Mean experience period of the participants in winter swimming amounted to 3.0 ± 1.9 years/seasons. In the investigated group, baseline body composition assessment was performed and baseline anthropometric traits were measured using a body composition analyzer (Tanita BC 418 MA) that employs bioelectrical impedance analysis (BIA). The obtained indices are presented in Table 1 and demonstrate normal values with reference to the participants' age and sex. Only body mass index (BMI) was slightly above the normal range, which indicates that the participants were pre-obese [23]. However, the levels of physical activity and aerobic fitness of the ice swimmers were high and average, respectively (Table 1). The level of physical activity during the last 7 days before the study was assessed using the International Physical Activity Questionnaire (IPAQ) [12]. Physical activity was expressed as MET min/wk (MET level × duration of physical activity per day/number of days of the week). MET means the baseline oxygen consumption and is 3.5 mL O_2/min/kg [12]. In turn, the participants' aerobic fitness was expressed by their maximum oxygen consumption (VO_{2max}), the best single indicator of aerobic fitness [24]. VO_{2max} was evaluated via an indirect method, the physical working capacity 170 (PWC_{170}) test [10]. The test was based on the performance of a 10-min exercise trial on a cycloergometer with individual loads in the first and sixth minute of the trial. Both loads were set in order not to exceed the heart rate of 170 beats per minute (bpm) during the whole trial. The result/index of the test was determined using the following formula: $PWC_{170} = P_1 + (P_2 - P_1)\cdot(170 - HR_1)/(HR_2 - HR_1)$, where $P_{1,2}$ = power of the first and second load (watts, W), $HR_{1,2}$ = heart rate at the end of the first and the second half osf the trial [10]. VO_{2max} demonstrates a strong positive correlation with the PWC_{170} index, therefore, it was determined graphically based on the index, according to Astrand–Ryhming nomogram [2]. VO_{2max} was expressed as mL O_2/min/kg. The men were asked to maintain their eating habits and physical activity immediately before or during the study period, that is, for 1.5 months. The study was approved by the Bioethics Committee at Nicolaus Copernicus University Ludwik Rydygier Collegium Medicum in Bydgoszcz, Poland (No.: KB 657/2012). The participants were informed about the study aims and the potential risks associated with the study, and signed informed consent forms.

Table 1
Characteristics of the study participants (mean ± SD).

Number of subjects	20
Age (years)	31.2 ± 6.3
BM (body mass, kg)	83.4 ± 14.0
BH (body height, cm)	180.7 ± 4.3
BSA (body surface area, m^2)	2.0 ± 0.1
BMI (body mass index, kg/m^2)	25.5 ± 3.7
BF (body fat, %)	17.8 ± 6.4
TBW (total body water, %)	60.2 ± 4.7
MM (muscle mass, %)	42.9 ± 3.7
IPAQ (physical activity, MET·min/week)	3571.2 ± 2646.0
VO_{2max} (aerobic fitness, mL/kg/min)	41.7 ± 6.7

Study design

In section 1 of the study (Mondays), the subjects performed a 30-min cycloergometer exercise test followed by a 10-min recovery in a gym in a sitting position (room temperature = 20 °C, RT). On Fridays, section 2, the participants, following an identical 30-min exercise test, recovered in a pool of ice-cold water at 3 °C for 5 min – an ICW bath excluding head immersion/to the mastoid line (the whole duration amounted to 10 min, including getting dressed). During both study sections, blood for laboratory assays was collected from the basilic vein two times: before the 30-min exercise test (baseline) and 40 min after the RT or ICW recovery intervention.

Aerobic exercise

The intensities and loads during the 30-min cycloergometer exercise test were determined for each participant by means of the PWC_{140} index that could be recalculated from the PWC_{170} index using a linear relationship between the power/load of exertion and HR between 120 bpm and 170 bpm for men aged 19–40 [10]. The rationale for the use of the PWC_{140} index resulted from the obtained parameters of physical activity and aerobic fitness, as well as from the authors' earlier own pilot studies (not published). Thus, the exercise test was conducted with a maximum HR of 153.0 ± 11.0 bpm (80.5% of maximum heart rate, HR_{max} = 205 − age/2) to obtain the power of 145.8 ± 23.1 W. HR exceeded 140 bpm because of the duration of the exercise test which was 3 times longer than the duration of the PWC_{140} test. According to the Borg Category-Ratio-10 (CR10) Scale, the exercise test was assessed by the participants as somewhat hard (4.0 ± 1.4) [6].

Body temperature

Before the exercise test (baseline), in the 30th minute of the exercise and 1 min after ICW immersion, oral (T1) and ear temperatures (T2), as well as forehead (T3), sternum (T4), and thigh skin temperatures (T5) were measured in the participants (Table 3). T1 was determined under the tongue using an electronic thermometer with a precision of 0.1 °C. T2 was obtained by means of an infrared ear thermometer with an accuracy of 0.2 °C. However, skin temperature: T3, T4 and T5 were measured using

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Table 2
The levels of lipid peroxidation products, the activities of antioxidant enzymes and the total antioxidant capacity in ice swimmers before and 40 min after the 30-min cycloergometer exercise test followed by the recovery at room temperature (RT) or in ice-cold water (ICW) (mean ± SD).

Oxidative stress indicator	Baseline 1*	Exercise/RT	Baseline 2*	Exercise/ICW
plTBARS [10^{-2} nmol MDA/mL]	36.0 ± 6.7	39.1 ± 5.8	37.9 ± 5.5	37.8 ± 7.0
erTBARS [nmol MDA/g Hb]	33.4 ± 8.1	32.3 ± 8.3	28.1 ± 7.1	30.4 ± 6.6
8-iso-PGF2α [10^2 pg/mL]	26.8 ± 2.9	25.9 ± 3.3	24.2 ± 2.9[a]	25.6 ± 3.2
4-HNE [mg/mL]	44.9 ± 13.6	49.6 ± 11.6	55.6 ± 15.0	50.6 ± 14.7
MDA [μmol/mg]	3.3 ± 0.6	3.4 ± 0.5	3.6 ± 0.5	3.5 ± 0.4
CAT [10^4 IU/g Hb]	54.3 ± 8.5	54.5 ± 8.2	60.0 ± 6.0	56.4 ± 7.4
SOD [10 U/g Hb]	78.0 ± 10.4	82.1 ± 10.4	85.9 ± 11.0	85.0 ± 14.7
TAC [10^{-1} mM UAE]	4.3 ± 0.7	4.3 ± 0.7	4.3 ± 0.8	4.3 ± 0.8

* The results obtained directly before the appropriate exercise test.
[a] Difference versus baseline 1 ($P < 0.05$); plTBARS, plasma thiobarbituric acid reactive substances; erTBARS, erythrocytic thiobarbituric acid reactive substances; 8-iso-PGF2α, 8-iso-prostaglandin F2α; 4-HNE, 4-hydroxynonenal; MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase; TAC, total antioxidant capacity.

Table 3
Body temperature of the study subjects before and in the 30th minute of the exercise test, as well as 1 min after ice-cold water (ICW) immersion (mean ± SD).

	T1	T2	T3	T4	T5
Baseline	35.9 ± 0.9	35.3 ± 0.4	33.5 ± 0.3	33.2 ± 0.2	29.9 ± 0.4
30th minute of exercise test	35.4 ± 0.3	36.6 ± 0.5**	n/d	n/d	n/d
1 min after ICW bath	35.9 ± 0.7	36.0 ± 0.5	30.5 ± 0.4***	22.9 ± 0.3***	20.2 ± 0.5***

*Statistically significant difference compared to the baseline (**$P < 0.01$, ***$P < 0.001$); T1 and T2: oral and ear temperature; T3, T4 and T5: forehead, sternum and thigh skin temperature.

an infrared thermal imaging camera with a 2% accuracy in the middle of the forehead, on the sternum and in the middle of the front of the right thigh. The distance between the camera and the subject was approx. 1 m. Before the temperature measurements, the subject's body was thoroughly dried, therefore only T1 and T2 were measured during the exercise test. All temperature measurements were performed at room temperature (approx. 20 °C).

Measurements

TBARS concentrations were determined in both plasma and erythrocytes using the spectrophotometric method described by Buege and Aust [7] and modified by Esterbauer and Cheeseman [9]. A plasma or hemolysate volume of 0.5 mL was mixed with 4.5 mL reaction mix containing 0.375% thiobarbituric acid (TBA) and 15% trichloroacetic acid (TCA) in 0.25 N HCl. Subsequently, the samples were incubated and centrifuged. The identification of TBARS was performed in supernatants and achieved via the measurement of absorbance at a wavelength of 532 nm versus the reaction mix incubated in the same conditions. TBARS consist mainly of MDA, therefore, the TBARS level in plasma was expressed as the MDA level per mL of plasma (10^{-2} nmol MDA/mL), while in erythrocytes, as the MDA level per g of hemoglobin (nmol MDA/g Hb).

The serum levels of 8-iso-PGF2α, 4-HNE and MDA were determined in enzymatic immunoassays using commercial ELISA kits. The quantity of lipid peroxidation products in unknown samples was determined by comparing their absorbance with a known standard curve. These assays involve reactions of lipid peroxidation products present in the investigated sample with specific antibodies bound on a 96-well plate and reading the absorbance of each well on a microplate reader using the wavelength of 450 nm. The concentrations of 8-iso-PGF2α, 4-HNE and MDA were expressed as 10^2 pg/mL, mg/mL and μmol/mg, respectively.

The CAT activity was assessed in erythrocytes using the Beers and Sizer method [4]. The principle of the method is based on a decrease in the absorbance of a hydrogen peroxide solution. H_2O_2 is decomposed by the enzyme, so the decrease in absorbance is directly proportional to the CAT activity in the solution. The CAT activity was expressed in 10^4 IU per g of hemoglobin. The SOD activity was also assayed in erythrocytes using the method by Misra and Fridovich [16]. Inhibition of oxidation of adrenaline to adrenochrome in alkaline environment was performed. This induced a change in the absorbance of the investigated solution. The SOD activity was expressed in 10 U per g of hemoglobin.

TAC was measured in blood serum using a commercial ELISA kit. The kit was based on a reduction of copper ions in an oxidated state of +2 to +1 by antioxidants present in the investigated sample. The amount of antioxidants was directly proportional to the concentration of copper +1 ions that were subjected to a reaction with a chromogen, forming colored compounds. Subsequently, the absorbance of the obtained solution was measured at $\lambda = 490$ nm and compared with the absorbance values on the calibration curve, which enabled the estimation of the antioxidant levels in the sample. TAC in the study sample was expressed as the concentration of uric acid equivalents (10^{-1} mM URE).

Statistical analysis

The results were subjected to statistical analysis using ANOVA test with post hoc analysis (Tukey's range test) or dependent *t*-test. Normal distribution was verified using the Kolmogorov–Smirnov test. The results are shown as means ± standard deviations. Changes at the level $P < 0.05$ were considered as statistically significant.

Results

No statistically significant differences were revealed in the concentrations of plTBARS, erTBARS, 8-iso-PGF2α, 4-HNE, and MDA, as well as in the CAT, SOD and GPx activities, along with the TAC level, in the subjects' blood before the exercise test compared to the 40th minute after the recovery both at RT and in ICW.

The baseline concentration of 8-iso-PGF2α in section 1 of the study (the exercise/RT combination) was higher in a statistically significant manner than in section 2 (the exercise/ICW

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combination) ($P < 0.05$; Table 2). The tendency towards higher TBARS concentrations in both blood plasma and erythrocytes was observed after the exercise/RT recovery compared to the exercise/ICW recovery ($P > 0.05$). In turn, an inverse tendency was found in the CAT and SOD activities in a similar comparison ($P > 0.05$).

A statistically significant negative linear correlation was found between the erTBARS concentration and the SOD activity in the 40th minute after the RT recovery preceded by the cycloergometer exercise test ($r = -0.571$, $P < 0.01$). A positive value of Pearson's r in section 2 before the exercise test was revealed between the CAT and SOD activities ($r = 0.469$, $P < 0.05$).

Discussion

The obtained results indicate that the aerobic exercise test and a short ice-cold water bath were not sufficiently strong stimuli to disrupt the oxidant–antioxidant balance in healthy ice swimmers. After the exercise, no statistically significant changes in the concentrations of lipid peroxidation products or changes in the activities of antioxidant enzymes, as well as in the TAC of the participants' blood serum, were revealed, even if the exercise test was followed by the ICW bath, although the baseline skin temperature was significantly different from the temperature measured 1 min after ICW immersion ($P < 0.001$; Table 3). The baseline ear temperature also changed in a statistically significant manner compared to the temperature determined in the 30th minute of the exercise test. In fact, physical activity increases the generation of reactive oxygen species which are mainly formed during the oxygen metabolism in the mitochondrial transport chain. The only exception are dynamic/strength exercises in which anaerobic processes predominate [25]. Increased ROS production occurs after such exercises following ischaemia/reperfusion, i.e. a reaction catalyzed by xanthine oxidase [25]. In turn, in the respiratory chain in which some oxygen is naturally subjected to incomplete reduction, intensified oxygen metabolism occurs leading to an increased generation of ROS [25]. Although ICW bathing also enhances the ROS production [19], no changes in the levels of the investigated parameters were observed following the exercise test associated with the ICW recovery. Only the tendency towards the lower erTBARS and plTBARS concentrations, as well as the higher CAT and SOD activities after the exercise test followed by ICW bath were noted in the blood of the ice swimmers compared to the exercise test followed by RT recovery ($P > 0.05$; Table 2). This may be a proof of the beneficial effect (an increase of antioxidant capacity) of the recovery in ice-cold water for maintaining the oxidant–antioxidant balance after physical exercise. Simultaneously, it was observed that the erTBARS concentration in the 40th minute after the exercise/RT recovery was negatively correlated with the SOD activity ($P < 0.01$). This is a proof of the proper functioning of the antioxidant system in the ice swimmers because TBARS contain mainly MDA, i.e. a secondary lipid peroxidation product [25]. However, SOD is active in the first/primary stage of free radical scavenging, viz. SOD eliminates superoxide anion forming hydrogen peroxide and an oxygen molecule [25]. Moreover, before the exercise/ICW recovery, a positive correlation between the CAT and SOD activities was shown ($P < 0.05$). The relation also indicates the proper functioning of the antioxidant system. The activity of catalase increases together with the activity of superoxide dismutase because hydrogen peroxide is automatically disproportionated by the catalase ($H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$) [25].

Increased antioxidant capacity is observed in physically well-trained people, primarily in professional athletes [17]. The study findings suggest that ice swimmers who regularly temper their bodies with cold water also have a more efficient antioxidant defense system, which has also been revealed by other authors [13,19]. However, in that case, it resulted in increased tolerance to physical exercise. The study revealed that both 5-min ice-cold water bath (3 °C) and 30-min aerobic exercise have practically no impact on the oxidant-antioxidant balance in healthy male ice swimmers. Nevertheless, the practical relevance relative to possible recovery effects is not clear, because the most recent reports demonstrate completely inconsistent effects of exposure to cold (cold water immersion, CWI; approx. 10 °C for 5–10 min) on the muscle function following strenuous exercise [11,18]. Thus, constant exposure of the human organism to low temperatures relative to possible recovery effects after physical exercise and the most suitable protocols for this purpose need to be verified by further studies separately for strength and aerobic exercise bouts.

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