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Whole-body Cryostimulation as an Effective Method of Reducing Oxidative Stress in Healthy Men

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. Whole-body cryostimulation (WBC) is the therapeutic exposure of the total human body (without underwear) to a very low temperature (below –100°C) for 120–180 s. Currently, WBC is used more frequently not only in the treatment of patients suffering from various diseases, but also by healthy people as a wellness method.
Objectives. The aim of this research is to evaluate the impact of WBC procedures on oxidative stress parameters in healthy men.
Material and Methods. The study involved 32 healthy male subjects who were randomly divided into 2 groups: 16 men exposed to WBC procedures with subsequent kinesiotherapy (WBC group) and 16 men exposed only to kinesiotherapy procedures (KT group). Depending on the group, the subjects were exposed to 10 daily WBC procedures lasting 3 min, with a subsequent 60-min of kinesiotherapy, or exclusively to kinesiotherapy. In subjects from both groups, a day before the beginning of a cycle of treatment and a day after its completion, the level of selected indicators of oxidative stress and non-enzymatic antioxidants, as well as the activity of antioxidant enzymes in serum, plasma and erythrocyte lysates were determined.
Results. In the WBC group subjects, we recorded a statistically significant decrease in the concentrations of most of the parameters of oxidative stress with an accompanying increase in plasma concentrations of non-enzymatic antioxidants (total antioxidant status and uric acid). We recorded no significant changes in the activities of antioxidant enzymes (plasma total superoxide dismutase (SOD) and its isoenzymes SOD-Mn and SOD-ZnCu, erythrocyte catalase, glutathione peroxidase and glutathione reductase).
Conclusions. The results we obtained confirmed that WBC decreases oxidative stress in healthy men (**Adv Clin Exp Med 2016, 25, 6, 1281–1291**).

Key words: oxidative stress, antioxidant enzymes, whole-body cryostimulation, healthy men, non-enzymatic antioxidants.

Whole-body cryostimulation (WBC) is the therapeutic exposure of the total human body (without underwear) to a very low temperature (below –100°C) for 120–180 s [1].

Until now, WBC has been used primarily in the treatment of inflammatory rheumatic diseases [1–3], multiple sclerosis [1], depressive syndrome [1, 4], and sports medicine [1, 5]. Currently,

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WBC is used more frequently as a wellness method [1].

Cryogenic temperatures cause several favorable physiological reactions, the effects of which are analgesic [1, 3], anti-inflammatory [1, 6–8], and promoting the circulatory effect [1, 9].

Cryogenic temperatures applied to the whole body, apart from the aforementioned effects, also have a significant influence on the psyche [4] and the endocrine system [10].

Very few studies published recently have shown that WBC may also have a beneficial influence on the human body by reducing oxidative stress in ankylosing spondylitis [11] and multiple sclerosis patients [12], and in healthy subjects [13, 14] and athletes [15].

Maintaining a prooxidant–antioxidant balance is very important for human health. Under conditions of a normal metabolism, the continuous formation of reactive oxygen species (ROS) is important for normal physiological functions like the generation of ATP, various catabolic and anabolic processes, and the accompanying cellular redox cycles. However, excessive generation of ROS can occur as a result of endogenous biological or exogenous environmental factors, such as chemical exposure, pollution, radiation, alcohol consumption and medications may cause oxidative damage, which accumulates over a lifetime, and has been implicated in aging and age-dependent diseases such as cardiovascular disease, cancer, neurodegenerative disorders, and other chronic conditions [16].

What is more, elevated levels of systemic oxidative stress may be found in healthy subjects. It may cause abnormal endothelial function, which may, in turn, cause an increased risk in the development of hypertension, atherosclerosis or other medical conditions [17]. It has been shown that lowering oxidative stress improves the endothelial function in healthy subjects [18].

Oxidative stress can also induce acute or chronic inflammation through the activation of multiple pathways. When oxidative stress appears as a primary disorder, inflammation develops as a secondary disorder and further enhances oxidative stress [19]. Therefore, taking into account current knowledge, it is important to reduce oxidative stress in healthy subjects.

Taking this data into account, the aim of this study is to evaluate the impact of 10 whole-body cryostimulation procedures on oxidative stress parameters in healthy men.

Material and Methods

Subjects

The study involved 32 non-smoking healthy male volunteers who were subjected to 2 different forms of wellness and divided randomly, with an allocation ratio of 1 : 1, into 2 groups: 16 men exposed to WBC procedures with subsequent kinesiotherapy (WBC group, mean age 45.19 ± 1.52 years) and 16 men exposed only to kinesiotherapy procedure (kinesiotherapy – KT group, control group, mean age 46.06 ± 1.44 years), with no significant difference in the mean age and Body Mass Index (BMI) between each group. Computer-generated random numbers were sealed in sequentially numbered envelopes, and the group allocation was independent of the time and person delivering the treatment. The physician (main coordinator) who allocated the patients to groups had 32 envelopes, each containing a piece of paper marked with either group (WBC or KT). The physician would select and open an envelope in the presence of a physiotherapist to see the symbol and would then direct the subject to the corresponding group. The demographic data of the study subjects are shown in Table 1.

The research protocol has been reviewed and approved by the Bioethical Committee of the Medical University of Silesia in Katowice (permission number: NN-6501-93/I/07), and all analyzed subjects gave their informed, written consent for inclusion in the trial. The trial was carried out in accordance with the Declaration of Helsinki.

Before the study each subject was examined by a physician to exclude any coexisting diseases as well as any contraindications for WBC procedures. Prior to the study a resting electrocardiogram was performed on all subjects, and blood pressure was measured in all the subjects before each session of cryostimulation [1]. All subjects were healthy volunteers who qualified for a routine complex treat-

Table 1. Demographic data of the study subjects (numerical values are expressed as means ± standard deviations SD)

Characteristic	WBC group (n = 16)	KT group (n = 16)	P-value
Age [years]	45.19 ± 1.52	46.06 ± 1.44	0.096
BMI [kg/m^2]	24.3 ± 4.1	23.8 ± 5.6	0.964
Smoking (yes/no)	0/16	0/16	–

ment called cryorehabilitation including WBC (treated as an assisting component) with subsequent kinesiotherapy or kinesiotherapy procedures only in order to obtain the wellness level. The subjects from the experimental group were not previously exposed to WBC. The subjects were asked to abstain from alcohol, drugs and any immunomodulators, immunostimulators, hormones, vitamins, minerals or other substances with antioxidant properties 4 weeks before the study, and they maintained the same diet and mode of physical activity during the trial. All subjects were also asked to refrain from the consumption of caffeine 12 h prior to each laboratory analysis.

Whole-body Cryostimulation and Kinesiotherapy Procedure

Depending on the group, the subjects were exposed to a cycle of WBC procedures lasting 3 min a day, with a subsequent 60 min of kinesiotherapy, or 60 min of only kinesiotherapy, for 10 consecutive days with a weekend break between.

The WBC procedures were performed in the Metrum Cryoflex cryochamber, which consists of 2 compartments: the antechamber and the proper chamber.

In the trial the temperature in the antechamber was –60°C, whereas in the proper chamber it reached –120°C. After a 30 s of the adaptation process in the antechamber, the subjects were exposed to cryogenic temperatures for 3 min in the proper chamber. During the WBC procedure, all subjects were dressed in swimsuits, cotton socks and gloves, and wooden shoes, and their mouths and noses were protected by surgical masks and their ears by ear protectors. All jewelry, glasses and contact lenses were removed before entry. During the WBC procedure the subjects were walking around the chamber without touching each other.

Immediately after leaving the cryochamber and changing clothes and shoes (for tracksuits and trainers), the subjects underwent 1-h long kinesiotherapy [1]. The program of kinesiotherapy consisted of exercises on stationary bikes lasting 20 min (5 min – 3.5 rate of perceived exertion (RPE), 4 min – 5 RPE, 2 min – 7 RPE, 4 min – 5 RPE, and 5 min – 3.5 RPE), treadmill (20 min walking at 4.0 mph) and whole body stretching exercises lasting 20 min. The procedure of kinesiotherapy was the same for all subjects in both groups. The subjects exercised under the close supervision of an exercise physiologist.

All subjects completed the study and no complications or side effects related to the WBC procedures or kinesiotherapy were observed.

Sample Collection

On the first day before the beginning of a WBC cycle and/or kinesiotherapy, and again on the first day after the end of a treatment cycle, whole blood samples were taken from all subjects, each time in the morning before the first meal.

Samples of whole blood (5 mL) were drawn from a basilic vein of each subject and then collected into tubes containing ethylenediaminetetraacetic acid (EDTA) (dose 2 mg/mL EDTA-K_3) and tubes with a clot activator. The blood samples were centrifuged (15 min, 3000 g, 4°C) and then the plasma and serum were immediately separated and stored at a temperature of –70°C, until biochemical analyses were performed. In turn, the red blood cells were rinsed with isotonic salt solution and then 10% hemolysates were prepared for further analyses.

Biochemical Analyses

In the plasma we determined the concentration of malondialdehyde (MDA), total antioxidant status (TAS) and the activity of the following antioxidant enzymes: total superoxide dismutase (SOD) and its isoenzymes, manganese-dependent (SOD-Mn) and zinc-copper-dependent (SOD-ZnCu).

In the serum we determined the activity of ceruloplasmin oxidase (CER), the concentration of protein sulfhydryl (PSH), uric acid (UA), lipofuscin (LF), oxidized-low density lipoprotein (ox-LDL), antibodies to oxidized-low density liporotein (ox-LDL ab) and total oxidant status (TOS).

In the lysates of the red blood cells we determined the activity of the following antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and the concentration of malondialdehyde (MDA).

Determination of Activity of Antioxidant Enzymes

The activity of superoxide dismutase (SOD-E.C.1.15.1.1) was determined by the Oyanagui method [20]. Enzymatic activity was expressed in nitrite unit (NU) in each mg of hemoglobin (Hb) or mL of blood plasma. One nitrite unit (1 NU) means a 50% inhibition of nitrite ion production by SOD in this method. SOD isoenzymes (SOD-Mn and SOD-ZnCu) were measured using potassium cyanide as the inhibitor of the SOD-ZnCu isoenzyme.

The activity of catalase (CAT-E.C.1.11.1.6.) was measured according to Aebi [21], with the use of hydrogen peroxide as a substrate and expressed in [IU/mg Hb].

The activity of glutathione peroxidase (GPx-E.C.1.11.1.9.) was assayed by Paglia and Valentine's kinetic method [22], with reduced glutathione (GSH) and t-butyl peroxide as a substrate. The activity of GPx was expressed as the quantity of μmol of a reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) used to recover reduced glutathione in 1 min, calculated for 1 g of hemoglobin [IU/g Hb].

The activity of glutathione reductase (GR-E.C.1.6.4.2) was assayed by Richterich's kinetic method [23], with the use of oxidized glutathione as a substrate, and it was determined as the quantity of μmol of NADPH used to recover GSH in 1 min, calculated to 1 g of hemoglobin [IU/g Hb].

Determination of Non--enzymatic Antioxidant Status

The plasma concentration of total antioxidant status (TAS) was determined by means of original diagnostic kits manufactured by Randox Laboratories Ltd. (The UK). The TAS concentration was expressed in nmol/L.

The serum concentration of protein sulfhydryl (PSH) was determined by Koster's method [24], using dithionitrobenzoic acid (DTNB). The PSH concentration was expressed in μmol/L.

The serum concentration of uric acid (UA) was determined by a colorimetric enzyme assay with the use of uricase [25] by means of the Cobas Integra 400 plus analyzer, and expressed as [mg/dL].

The serum activity of the ceruloplasmin oxidase (CER) was measured with the use of the p-phenylenediamine kinetic method by Richterich [23]. Enzymatic activity was expressed in mg/dL after a calibration with pure ceruloplasmin isolated from a healthy donor serum pool.

Determination of Oxidative Stress Markers

The intensity of lipid peroxidation in the plasma and the erythrocytes was measured fluorimetrically as a concentration of thiobarbituric acid-reactive substances (TBARS) after the reaction of the sample with sodium dodecyl sulfate, acetic acid, 2-thiobarbituric acid and a butanol-pyridine mixture, according to Ohkawa [26]. The butanol-pyridine layer was measured fluorometrically (at 515 nm (excitation) and at 552 nm (emission) by means of a spectrofluorimeter from Shimadzu (Rydalmere, Australia). Tetraethoxypropane was used as the standard. The TBARS concentrations were expressed as malondialdehyde (MDA) equivalents in μmol/L in plasma or nmol/g Hb in erythrocytes.

The serum total oxidant status (TOS) was determined with the method described by Erel [27]. The method is based on the oxidation of Fe^{2+} ions to form Fe^{3+} ions by oxidizing agents (lipid peroxides and hydrogen peroxide) contained in the sample. The intensity of the color complex formed between Fe^{3+} ions and xylenol orange was measured spectrophotometrically. The TOS concentration was expressed in μmol/L.

The serum concentration of oxidized-low density lipoprotein (ox-LDL) was measured with the use of an ELISA kit (Biomedica, Poland). The ox-LDL concentration was expressed in [ng/mL].

The serum concentration of antibodies to oxidized-low density lipoprotein (ab-ox-LDL) was measured with the use of an ELISA kit (Biomedica, Poland). The ab-ox-LDL concentration was expressed in nU/mL.

The serum concentration of lipofuscin (LF) was determined by the Tsuchida method [28]. Serum was added to a mixture of ethanol-ether, 3 : 1 (v/v) and centrifuged. The fluorescence intensity of the dissolved precipitate was determined by an LS45 spectrofluorimeter (manufactured by PerkinElmer), at a wavelength of 345 nm (absorbance) and 430 nm (emission). The values were expressed in relative units (relative lipid extract fluorescence, RF), where the RF value of 100 corresponded to the fluorescence of a solution of 0.1 mg/mL quinidine sulfate in 0.1 N sulfuric acid. The LF concentrations were shown in RF/mL.

The oxidative stress index (OSI), an indicator of the degree of oxidative stress, was expressed as the ratio of total oxidant status (TOS) to total antioxidant status (TAS) in arbitrary units [29].

Statistical Analyses

Statistical analyses were undertaken using the statistical package of STATISTICA 10 PL software. For each parameter the indicators of the descriptive statistics were determined (mean value and standard deviation SD). The normality of the data distribution was checked using the Shapiro–Wilk test, while the homogeneity of the variance was verified with use of Levene's test. Next, a two-way analysis of variance was performed in order to verify if there is an effect of the main factor; WBC with subsequent kinesiotherapy or kinesiotherapy procedure on the studied laboratory parameters. Afterwards, we found a positive result of analysis of variance in *post-hoc* tests. In order to compare the differences between the initial values of particular laboratory parameters and their values after the end of a cycle of treatment procedures in both groups of subjects, a dependent sample Student's t-test was used when the homo-

geneity of variances and normality of distribution were fulfilled; otherwise a Wilcoxon signed-rank test was used. In order to compare the differences of the values of particular laboratory parameters between both groups of subjects an independent (un-paired) sample Student's t-test or Mann-Whitney U test was used, depending on the homogeneity of variances and normality of data distribution.

Differences at a significance level of $p < 0.05$ were considered as statistically important.

Results

In the WBC group subjects, who underwent a 10-day long cycle of WBC procedures with subsequent kinesiotherapy, no significant changes in the activity of plasma total SOD and its izoenzymes SOD-Mn and SOD-ZnCu, as well as in the activity of erythrocyte SOD, CAT, GPx and GR were observed.

In turn, in the subjects from the group who underwent a cycle of kinesiotherapy only without previous cryostimulation procedures, after the completion of treatment, the activity of erythrocyte SOD and GR decreased significantly, by 13% and 24%, while the activity of erythrocyte CAT increased significantly by 10% and the activity of erythrocyte GPx did not change significantly in comparison to initial values. Moreover, after the completion of the kinesiotherapy cycle, the activity of erythrocyte CAT was significantly higher, in comparison to the WBC group. Similarly, as in the WBC group subjects, no statistically significant changes in the activity of plasma SOD and its isoenzymes SOD-Mn and SOD-ZnCu as well as erythrocyte GPx were observed after the completion of treatment, though the activity of erythrocyte GPx was significantly lower, by 31.6%, in comparison to the WBC group (Table 2).

In the WBC group subjects, after the completion of a cycle of cryostimulation procedures with subsequent kinesiotherapy, a statistically significant increase in plasma concentration of TAS, by 96%, and a significant decrease in serum concentration of UA, by 10% were observed, in comparison to initial values. In contrast, in the KT group subjects, after the completion of the kinesiotherapy cycle, no significant changes in the plasma concentration of TAS and UA were noticed. In the case of TAS the difference prior to post-treatment values in the WBC group was significantly higher in comparison to the KT group.

Further, in the subjects from both groups the serum concentration of PSH and the activity of CER did not change significantly after the completion of treatment, in comparison to initial values (Table 3).

In the WBC group subjects, most of the markers of oxidative stress decreased significantly after the completion of the treatment in comparison to initial values. We observed a statistically significant decrease in plasma concentration of MDA by 14%, serum concentration of TOS by 22%, serum concentration of anti-ox-LDLab by 16%, serum concentration of LF, by 12% as well as OSI index by 57%. In turn, the concentration of erythrocyte MDA and the serum concentration of ox-LDL did not change significantly in the subjects from this group.

In the KT group no significant changes in the parameters of oxidative stress were observed after the completion of the treatment, in comparison to the initial values before the beginning of the kinesiotherapy cycle, though the concentration of erythrocyte MDA was significantly higher in comparison to the WBC group. For LF, TOS and OSI the differences prior to post-treatment values in the WBC group were significantly higher in comparison to the KT group (Table 4).

Discussion

The WBC group saw a significant increase in the concentration of plasma TAS and its main component UA after ten whole-body cryostimulation procedures, with subsequent kinesiotherapy. On the other hand, we noticed no changes in the activity of antioxidant enzymes, as well as a significant decrease in the level of estimated oxidative stress markers such as: plasma concentration of MDA, LPS, ox-LDL-Ab, TOS and OSI index in the same group. A lack of significant changes in the activity of antioxidant enzymes and a significant increase in the concentration of non-enzymatic antioxidants, in addition to a significant decrease in the level of oxidative stress parameters after the completion of the WBC group proves that 10 WBC procedures do not intensify the generation of oxidative stress and increase non-enzymatic antioxidant status.

It is noteworthy that in our study we estimated the influence of WBC procedures as a method of wellness, performed for the first time in a cryochamber with cold retention, where the coolant is liquid air. To our knowledge, our study is the first report that assesses the influence of WBC with subsequent kinesiotherapy on the antioxidant status in healthy people through a simultaneous assessment of the activity of the enzymatic system (both in the plasma and in the erythrocytes), as well as in the non-enzymatic system of the plasma,

Table 2. Activities of antioxidant enzymes (mean value ± standard deviation SD) in healthy subjects before and after the completion of a cycle of 10 whole-body cryostimulation procedures with subsequent kinesiotherapy (WBC group) or a cycle of only 10 kinesiotherapy procedures (KT group), with statistical analysis; (p) – plasma; (e) – erythrocyte lysates; Δ – difference prior to post-treatment

Parameters		**WBC group**	**KT group**	**P-value**
Total SOD (p) [NU/mL]	before	12.2 ± 2.20	11.2 ± 1.93	0.173
	after	11.8 ± 3.04	11.6 ± 3.26	0.811
	P*	0.918	0.679	
	Δ	–0.35 ± 3.28	0.40 ± 3.82	0.554
SOD-Mn (p) [NU/mL]	before	5.31 ± 2.02	4.50 ± 1.41	0.204
	after	6.22 ± 1.40	5.16 ± 2.19	0.115
	p*	0.115	0.179	
	Δ	0.91 ± 2.00	0.65 ± 2.00	0.715
SOD-ZnCu (p) [NU/mL]	before	6.88 ± 1.12	6.72 ± 1.96	0.782
	after	5.84 ± 1.75	6.60 ± 2.92	0.381
	p*	0.121	0.918	
	Δ	–1.04 ± 2.42	–0.12 ± 3.05	0.353
Total SOD (e) [NU/mgHb]	before	89.7 ± 7.80	114.0 ± 20.80	< 0.001
	after	91.4 ± 6.10	98.5 ± 17.40	0.142
	p*	0.796	0.002	
	Δ	1.67 ± 8.86	–15.3 ± 12.70	< 0.001
CAT (e) [IU/mg Hb]	before	315.0 ± 47.8	385.0 ± 92.6	0.014
	after	341.0 ± 57.2	425.0 ± 98.3	0.007
	P*	0.255	0.045	
	Δ	26.1 ± 80.2	39.9 ± 80.0	0.629
GPx (e) [IU/g Hb]	before	26.5 ± 2.18	23.8 ± 7.20	0. 160
	after	27.9 ± 2.23	21.2 ± 3.71	< 0.001
	P*	0.098	0.179	
	Δ	1.33 ± 2.74	–2.59 ± 7.99	0.079
GR (e) [IU/g Hb]	before	1.47 ± 0.31	1.49 ± 0.53	0.856
	after	1.38 ± 0.25	1.14 ± 0.61	0.157
	P*	0.427	0.010	
	Δ	–0.08 ± 0.41	–0.35 ± 0.4	0.084

p – statistical significance of differences between both groups of subjects; p* – statistical significance of differences between values before and after treatment in particular groups of subjects.

in correlation with the indicators of the intensity of oxidative stress.

Only a few papers have estimated the influence of WBC on the prooxidant-antioxidant balance in healthy subjects, but their results are not unique, as the authors used different procedures of cryostimulation and kinesiotherapy, and they estimated only a level of selected parameters of the erythrocyte antioxidant defense system and oxidative stress markers. The next important point is that most of the previous studies did not have a control group, in which the subjects were exposed solely to kinesiotherapy. Consequently, it is difficult to answer whether the observed results were related to the action of WBC or kinesiotherapy, which is an obligatory component of cryorehabilitation procedures.

Table 3. Concentration of non-enzymatic antioxidants (mean value ± standard deviation SD) in healthy subjects before and after the completion of a cycle of 10 whole-body cryostimulation procedures with subsequent kinesiotherapy (WBC group) or a cycle of only 10 kinesiotherapy procedures (KT group), with statistical analysis; (p) – plasma; (s) – serum; Δ – difference prior to post-treatment

Parameters		**WBC group**	**KT group**	**P-value**
TAS (p) [nmol/L]	before	0.89 ± 0.57	1.59 ± 0.45	0.001
	after	1.74 ± 0.77	1.47 ± 0.65	0.294
	P*	0.006	0.569	
	Δ	0.85 ± 0.98	–0.12 ± 0.57	< 0.001
PSH (s) [μmol/L]	before	582.6 ± 120.1	481.4 ± 126.8	0.028
	after	536.7 ± 64.9	451.4 ± 137.0	0.035
	P*	0.109	0.088	
	Δ	–45.9 ± 99.0	–30.1 ± 163.2	0.744
UA (s) [mg/dL]	before	6.57 ± 1.85	5.61 ± 0.86	0.074
	after	6.33 ± 3.46	5.31 ± 1.24	0.281
	P*	0.023	0.143	
	Δ	–0.24 ± 3.57	–0.30 ± 0,88	0.947
CER (s) [mg/dL]	before	57.47 ± 13.82	50.69 ± 12.29	0.153
	after	48.99 ± 19.62	47.25 ± 10.69	0.758
	P*	0.326	0.328	
	Δ	–8.48 ± 25.19	–3.44 ± 15.85	0.505

p – statistical significance of differences between both groups of subjects; p* – statistical significance of differences between values before and after treatment in particular groups of subjects.

Lubkowska et al. [30] showed that a single session of WBC (temperature: –130°C, time: 3 min, two-step cryochamber, liquid nitrogen coolant) could induce disturbances in prooxidant-antioxidant balance, in the form of lowering TOS and a temporary decrease in TAS, with a subsequent elevation of those parameters on the next day, resulting in an intensification of oxidative stress.

In another study by this team [31], healthy men were exposed to a single WBC session (temperature: –130° C, time: 3 min, two-step cryochamber, liquid nitrogen coolant) without subsequent kinesiotherapy. The authors observed a significant increase in GPx and GR activities, with a simultaneous decrease in CAT and glutathione S-transferase activities. A significant increase in the concentration of glutathione, uric acid, albumins and extraerythrocyte hemoglobin was also observed in the serum of the subjects. The authors concluded that a single stimulation with cryogenic temperatures results in oxidative stress in a healthy body, but the level of this stress is not very high. However, the authors did not perform studies in a whole cycle of cryostimulation so those studies are insufficient in comparison to the results presented in this paper.

It has been suggested that repeated exposure to cryogenic temperatures may cause adaptative changes in the form of an increase in antioxidant status and antioxidant enzyme activity, resulting in the formation of a prooxidant-antioxidant balance at a higher level, assisting in an anti-inflammatory effect and protecting tissues against an increased generation of reactive oxygen species and oxidative stress caused by training [15].

Miller et al. [32] observed that 10 sessions of WBC (temperature: –130°C, time: 3 min, without subsequent kinesiotherapy) may reduce the generation of different reactive oxygen and nitrogen species in healthy subjects, due to an increase in the plasma level of SOD, UA and TAS. The authors also found a significant increase in the thiobarbituric acid reactive substance (TBARS) concentration as a marker of lipid peroxidation in healthy men who underwent WBC, while in women the concentration of TBARS did not change significantly after the completion of WBC procedures. However, the mean value of TBARS concentration in all subjects after the completion of WBC procedures was higher in comparison to the control group. One must emphasize that in this study only four parameters were

Table 4. Values of oxidative stress parameters (mean value ± standard deviation SD) in healthy subjects before and after the completion of a cycle of 10 whole-body cryostimulation procedures with subsequent kinesiotherapy (WBC group) or a cycle of only 10 kinesiotherapy procedures (KT group), with statistical analysis; (p) – plasma; (s) – serum; (e) – erythrocyte lysates; Δ – difference prior to post-treatment.

Parameters		WBC group	KT group	P-value
MDA (p) [μmol/L]	before	2.37 ± 0.39	2.30 ± 0.52	0.669
	after	2.04 ± 0.52	2.39 ± 0.52	0.072
	P*	0.039	0.057	
	Δ	–0.33 ± 0.58	0.09 ± 0.70	0.079
MDA (e) [nmol/L]	before	0.14 ± 0.02	0.17 ± 0.03	0.001
	after	0.13 ± 0.01	0.17 ± 0.05	0.004
	P*	0.070	0.999	
	Δ	–0.01 ± 0.02	0.00 ± 0.05	0.487
TOS (s) [μmol/L]	before	18.68 ± 6.71	10.12 ± 3.28	< 0.001
	after	14.54 ± 5.78	11.97 ± 6.71	0.254
	P*	0.047	0.485	
	Δ	–4.13 ± 9.18	1.85 ± 7.80	0.050
ox-LDL (s) [ng/mL]	before	180 ± 51.1	153 ± 52.8	0.159
	after	156 ± 37,0	141 ± 32.0	0.237
	P*	0.121	0.176	
	Δ	–24.2 ± 60.9	–12.4 ± 33.4	0.505
anti-oxLDLab (s) [nU/mL]	before	336.0 ± 96.5	368.0 ± 237.0	0.627
	after	281.0 ± 124.0	313.0 ± 191.0	0.578
	P*	0.013	0.605	
	Δ	–55.1 ± 110.0	–54.6 ± 255.0	0.994
LF (s) [RF/mL]	before	787 ± 1 36	735 ± 78.1	0.194
	after	696 ± 127	779 ± 110	0.056
	P*	0.021	0.148	
	Δ	–91.4 ± 151	44.3 ± 103	0.006
OSI (p/s) [arbitrary unit]	before	25.18 ± 13.78	6.84 ± 3.03	< 0.001
	after	10.79 ± 8.62	10.79 ± 9.02	0.998
	P*	0.007	0.196	
	Δ	–14.40 ± 17.62	3.96 ± 8.73	0.001

p – statistical significance of differences between both groups of subjects; p* – statistical significance of differences between values before and after treatment in particular groups of subjects.

estimated: the activity of one antioxidant enzyme (SOD), the concentration of 2 non-enzymatic antioxidants (TAS and UA), in addition to the concentration of one marker of oxidative stress (TBARS). Furthermore, the subjects did not undergo kinesiotherapy, which is an obligatory component of routine WBC procedures. That is why we would like to emphasize that results presented in this paper contain much more data, and the studies may provide innovative and complementary conclusions.

In another study [14], the authors showed that the activity of antioxidant enzymes in healthy men depends on the number of WBC procedures. After 10 sessions (temperature: –130°C, time: 3 min) the

UA concentration was reduced, subsequently with a significant increase above the level prior to cryostimulation, after the completion of 20 sessions. The activity of individual antioxidant enzymes depended on the exposure time. A cycle of 10 sessions of WBC resulted in insignificant changes in the activity of SOD, GPx and GR, though in the case of SOD and GPx a distinct downward trend was observed. After the first 10 sessions, a marked increase was seen in CAT activity and in the level of glutathione, both reduced and oxidized, without any accompanying changes occurring in the ratios of GSH : GSSG and SOD : CAT. Additionally, after the prolongation of the WBC cycle to 20 sessions, the CAT activity returned to baseline, but the SOD activity significantly increased, which resulted in a significant increase in the SOD : CAT ratio. The continuing downward trend in GPx activity resulted in its significant reduction compared to the baseline. Consequently, the plasma concentration of 8-isoprostane (the best available biomarker of oxidative stress and lipid peroxidation status *in vivo*) increased significantly after both 10 and 20 sessions of WBC. The results obtained by the authors suggested that WBC intensifies oxidative stress and causes an accompanying decrease in antioxidant enzyme activity after 10 sessions with a subsequent compensatory increase after the completion of a cycle of 20 sessions.

In the study performed by Duque et al. [13], a significant increase was seen in the TAS value in healthy men at the end of a cycle of 45 procedures of WBC (temperature: –110°C, time: 2 min, coolant liquid nitrogen) performed 3 times a week. Yet, the standard protocol of WBC for patients most frequently used contains 10 procedures with subsequent kinesiotherapy every day.

The differences in the results of various studies may be related to the type of cryochamber being used and the coolant medium, in addition to the time of exposure to cryogenic temperatures and the length of kinesiotherapy time. All of the previously mentioned studies were performed in a closed cryochamber also referred to as a "Wroclawski type" cryochamber, or in a two-step cryochamber cooled by liquid nitrogen. In the "Wroclawski type" cryochamber the temperatures were not stable during the procedure [1].

In our KT group subjects, no significant changes in the level of oxidative stress parameters were observed. However, a significant drop in the activity of SOD and GR and an increase in the activity of CAT could be most likely a consequence of an increased consumption of SOD and GR due to the intensification of ROS generation during kinesiotherapy procedures. Decreased GR activity may be associated with the accumulation of glutathione disulfide (GSSG) [33]. It seems that the antioxidant enzymes do play a significant role in the prevention of the development of oxidative stress during kinesiotherapy procedures, though in the study [12] kinesiotherapy had no effect on CAT activity in patients with multiple sclerosis, but the kinesiotherapy procedures lasted only 20–30 min.

In recent years some studies have indicated that physical exercise can create oxidative stress [34]. During exercise, the VO_2 uptake is higher than during rest because of the increasing energy demand in many tissues, mainly in muscles, that results in elevated ROS generation [35].

Our results prove that WBC is a beneficial method which allows subjects who underwent kinesiotherapy or other physical exercise to decrease oxidative stress and keep the prooxidant-antioxidant balance at a safe level.

The present study has some limitations. First, the study did not provide a long-term follow-up (at least 3 months), and thus we do not know how long the beneficial effect of WBC with subsequent kinesiotherapy would be maintained after the completion of a WBC cycle. Second, a cycle of WBC with subsequent kinesiotherapy consisted of only 10 procedures. A greater number of procedures (e.g. 20–30) could probably increase the treatment effect as was reported in the literature [14, 36].

Further studies are necessary to estimate whether the number of WBC procedures and the type of cryochamber may have an influence on the prooxidant-antioxidant balance in subjects exposed to cryostimulation. Also, the standardization of exposure times and the number of treatments during each cryostimulation cycle could improve the comparability of the results obtained by different research teams. It should be underlined that the results presented in this paper contain much more data than other research and the studies performed may provide complementary conclusions not only for healthy people but, above all, for the patients.

The authors concluded that whole-body cryostimulation decreases oxidative stress in healthy men.

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