

The effect of a series of whole-body cryotherapy treatments on the activity of antioxidant enzymes in healthy women and women with multiple sclerosis

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The study aimed to compare the effect of a series of 20 sessions of whole-body cryotherapy (WBC) on the level of CAT, GPx and SOD in women with multiple sclerosis and healthy women. The consent of the Bioethics Committee at the Regional Medical Chamber in Krakow was obtained, and the trial was registered in the Australian New Zealand Clinical Trials Registry. Thirty people took part in the study: the study group (MS) and the control group (CONT). All subjects participated in 20 cryotherapy sessions. Venous blood was collected for analysis before the WBC session and after 20 sessions. There were no changes in the examined parameters (CAT, GPx, SOD) after using WBC in the MS or control groups. There were also no differences between the groups in the first or the last study. There were no adverse changes in the parameters tested – WBC appeared to be a safe form of therapy, although the mechanisms of hypothermic protection are not fully understood.

Key words: whole-body cryotherapy, multiple sclerosis, oxidative stress, CAT, GPx, SOD

INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune, inflammatory disease of the central nervous system characterized by demyelination, chronic inflammation, damage to gray matter neurons and axons, and oxidative stress. The cause of the disease is unknown but multifactorial. Many studies suggest that inflammation and oxidative stress may be one of the sources or consequences of the disease resulting from loss of the antioxidant/oxidative balance (Glass et al., 2010; Ferreira et al., 2013; Gray et al., 2014). Oxidative stress is the hallmark of neurodegeneration (Miller et al., 2011a).

In order to combat the harmful effects of free oxygen radicals, cells contain antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) (Siems et al., 1999; Zorov et al., 2014). CAT is an intracellular antioxidant enzyme; it is especially important in the case of the limited availability of glutathione and plays a significant role in the development of tolerance to cellular oxidative stress (Dringen et al., 2005; Schreibelt et al., 2007). GPx is a family of selenium-containing enzymes that detoxifies cellular organic peroxides and hydrogen peroxide through the oxidation of two glutathione molecules (Schreibelt et al., 2007; Herbet et al., 2007). Multiple studies have found that eryth-

rocyte GPx activity was decreased in MS patients compared to healthy controls (Groen et al., 2016). SOD provides the first line of defense against oxidative stress. Various studies have suggested that SOD may play an important role in neurodegeneration and neuritis (Schreibelt et al., 2007; Johnson and Giulivi, 2005). The increase in oxidative stress in the early stages of MS may be compensated by SOD, but SOD's antioxidant mechanisms gradually fail as the disease progresses, leading to ongoing neuronal damage. This is confirmed by the negative correlations between SOD erythrocyte activity and many disease markers of severity (duration of disease, burden of lesions, and Expanded Disability Status Scale – EDSS – scores) (Groen et al., 2016; Ljubisavljevic et al., 2014). Some studies have shown that SOD levels were decreased in MS (Emamgholipour et al., 2015), while others showed that SOD levels were increased in MS (Lee et al., 2004; Gironi et al., 2014).

The inflammatory environment in demyelinating lesions leads to the generation of oxygen- and nitrogen-free radicals as well as proinflammatory cytokines, which contribute to the development and progression of the disease. Inflammation can lead to oxidative stress and vice versa. Thus, oxidative stress and inflammation are involved in a self-perpetuating cycle (Yevgi and Demir, 2021). Cryotherapy treatment is implemented in diseases that involve the participation of reactive oxygen species (ROS). Neutralization of oxidative stress is considered to be a key mechanism that can explain the positive impact of cryotherapy (Skrzep-Poloczek et al. 2017). Cryotherapy of the whole body combined with kinesiotherapy improved the lipid profile and reduced oxidative stress in healthy people (Stanek et al., 2019), and in people with ankylosing spondylitis, it had a positive effect on reducing the clinical activity of the disease as measured by the BASDAI method (Bath Ankylosing Spondylitis Disease Activity Index) (Straburzyńska-Lupa et al. 2018). Previous research on the effect of whole-body cryotherapies (WBCs) on oxidative stress in people with MS showed that the treatment session had either no effect on the activity of CAT and SOD (Miller et al., 2010; 2011a) or it increased the activity of SOD and CAT (but together with additional supplementation) (Miller et al., 2011b) or raised only SOD1 (Bryczkowska et al., 2018). These studies used a different number of treatments and combined WBC treatments with other types of interventions.

The aim of the present study was to compare the effect of a series of 20 sessions of WBC on the levels of CAT, GPx, and SOD in women with MS and healthy women. It was hypothesized that WBC would improve the antioxidant capacity of the body in healthy people and

those with MS, measured by the following indicators: CAT, GPx, and SOD.

METHODS

Participant characteristics

The study (controlled and prospective) was conducted in accordance with the Helsinki Declaration of the World Medical Society, with consent from the Bioethics Committee at the Regional Medical Chamber in Krakow (87/KBL/OIL/2018; 08/05/2018) and registered in the Australian New Zealand Clinical Trials Registry (ACTRN12620001142921; 02/11/2020). Participants passed a medical qualification (neurologist and rehabilitation physician) before participating in the project.

To take part in the study, volunteers had to have been diagnosed with MS (McDonald's review criteria), obtain the Extended Disability Status Scale (EDSS): from 0 to 6.5, be a woman, be aged 30–55 years, and sign a consent to participate in the study.

Volunteers could not have contraindications to WBC (i.e., pregnancy, severe hypertension (BP>180/100), acute or recent myocardial infarction, unstable angina pectoris, arrhythmia, symptomatic cardiovascular disease, cardiac pacemakers, peripheral arterial occlusive disease, venous thrombosis, acute or recent cerebrovascular accident, uncontrolled seizures, Raynaud's Syndrome, fever, tumor disease, symptomatic lung disorders, bleeding disorders, severe anemia, infection, cold allergy, and acute kidney and urinary tract diseases); change diets during the project or in the period of 3 months before; or take part in other forms of treatment or physical activity during the project or in the period of 3 months before.

Thirty people took part in the study: 15 people with MS and 15 healthy people (without neurological diseases and other chronic diseases). All subjects participated in 20 cryotherapy sessions. The characteristics of the study and control groups are presented in Table 1.

Analysis of biochemical blood indices

For the analysis of blood indicators, venous blood was collected twice – before the start of WBC procedures (Study 1) and after a series of 20 WBC sessions (Study 2). Blood was collected in tubes with EDTA in the morning from the cephalic, fallen, or medial vein. The blood was centrifuged at 1,000 g for 10 min at 4°C to separate plasma and erythrocytes, which were then washed three times with cold (4°C) saline and kept frozen at -80°C until analyzed for the activity of an-

Table 1. Characteristics of the control group and the experimental group.

Characteristics	Study group (MS)	Control group (CONT)	
Age [years]	41.53 ± 6.98	38.47 ± 6.00	
Body height [cm]	165.93 ± 6.53	169.4 ± 5.79	
Body weight [kg]	66.75 ± 16.78	72.35 ± 13.85	
Body mass index [kg/m ²]	24.18 ± 5.68	25.22 ± 4.81	
Fat [%]	33.26 ± 7.45	30.47 ± 6.65	
Fat [kg]	23.31 ± 11.40	22.82 ± 8.99	
Fat-free mass [kg]	43.45 ± 5.68	49.55 ± 5.90	
Total body water [kg]	31.83 ± 4.21	36.28 ± 4.32	
EDSS score	3.03 ± 1.67	-	
Disease duration [years]	11.00 ± 6.49	-	
Disease course [%]	Primary progressive	13.33	-
	Relapsing-remitting	86.67	-
Occurrence of relapses [%]	Several times a year	6.67	-
	Once a year	20.00	-
	Every few years	60.00	-
	No relapse, MS progresses	13.33	-
Occurring disorders [%]	Spasticity	40.00	-
	Tremor	6.67	-
	Excessive fatigue	80.00	-
	Blurred vision	20.00	-
	Paresthesia	46.67	-
	Balance disorders	46.67	-
	Mood disorders	53.33	-
Pharmacological treatment [%]	Bladder dysfunction	33.33	-
	Immunomodulating agents	67.67	-
	Steroid agents	33.33	-
	None	6.67	-
	Low-dose naltrexone	6.67	-

tioxidant enzymes: catalase (CAT, EC 1.11.1.6, Aebi's method (Aebi, 1984)); glutathione peroxidase (GPx, EC 1.11.1.9, the commercial RANSEL RS504 kit by Randox, UK); and superoxide dismutase (SOD, EC 1.15.1.1, the commercially available RANSOD SD125 kit by Randox, UK). Biochemical assays were performed by a laboratory certified as meeting the requirements of PN-EN ISO 9001:2015, in line with the recommendations of the testing kit manufacturers.

Description of the intervention

WBC treatments were performed daily from Monday to Friday (15:00–17:00) in a Wrocław-type cryochamber where liquid nitrogen was used to cool the refrigerant. The specifications were atrium temperature: -60°C; chamber temperature: -120°C; and WBC duration: 3 min. In total, everyone received 20 treatments.

The women entering the cryochamber were dressed in bathing suits, high woolen socks and boots with high wooden soles, gloves, headgear, and a mask. During the procedure, visual contact with the patients was maintained through thermal windows. The procedure was additionally monitored by a camera. The cryochamber was equipped with the oxygen content, humidity and temperature of both the atrium and the proper chamber. The concentration of oxygen in the air of the cryochamber was kept constant at 21–22% and continuously controlled. The conditions inside the chamber were the same during all treatments in the series. A medical rehabilitation doctor was present at the Rehabilitation Center throughout the treatment.

After each treatment, there was a warm-up for each patient on a cycle ergometer (Kettler Corsa) without resistance for 15 min – controlled and supervised by a physiotherapist.

Statistical analysis

In order to determine the sample size, the formula for the minimum sample size was used, in which the

confidence interval was 95%, the fraction size of 0.5 and the maximum error of 5% was assumed. Descriptive statistics were determined for the mean (\bar{x}) as well as standard deviation (SD). The normality of distributions was verified with the Shapiro-Wilk test. Data distribution analysis was performed using parametric tests – the Student's t-test for dependent samples within the group and the same test for independent samples performing comparisons within the groups. The applied tests verified two-sided hypotheses. Analysis of variance was performed for repeated measurements with regard to the following effects: group, time, and multivariate factors group*time. The analyses were performed with the use of the Statistica 13 package (Tibco Software Inc., USA).

RESULTS

There were no changes in the examined parameters after the use of WBC in the MS group or in the control group. There were also no differences between the groups in either the first or the last study (Table 2-5, Fig. 1-3).

Table 2. CAT, GPx and SOD in the experimental group (MS) and in the control group (CONT) before (Study 1) and after (Study 2) the intervention sessions.

Parameter		MS	CONT	(p)
CAT [U/g Hb]	Study 1	176.57 ± 42.95	163.41 ± 31.95	0.349
	Study 2	169.35 ± 39.07	190.56 ± 50.15	0.207
	(p)	0.682	0.070	
GPx [U/g Hb]	Study 1	1317.20 ± 259.73	1250.81 ± 146.82	0.396
	Study 2	1342.19 ± 302.72	1238.92 ± 126.08	0.233
	(p)	0.627	0.695	
SOD [U/g Hb]	Study 1	49.47 ± 4.81	49.26 ± 9.84	0.942
	Study 2	48.38 ± 6.98	50.65 ± 6.42	0.362
	(p)	0.527	0.586	

Table 3. CAT, GPx and SOD in the experimental group (MS) before (Study 1) and after (Study 2) the intervention sessions.

Parameter	Group / Study			
	MS / Study 1		MS / Study 2	
	Shapiro-Wilk (W)	(p)	Shapiro-Wilk (W)	(p)
CAT [U/g Hb]	0.908	0.128	0.923	0.215
GPx [U/g Hb]	0.934	0.315	0.923	0.220
SOD [U/g Hb]	0.926	0.832	0.943	0.586

Table 4. CAT, GPx and SOD in the control group (CONT) before (Study 1) and after (Study 2) the intervention sessions.

Parameter	Group / Study			
	CONT / Study1		CONT / Study 2	
	Shapiro-Wilk (W)	(p)	Shapiro-Wilk (W)	(p)
CAT [U/g Hb]	0.897	0.085	0.922	0.209
GPx [U/g Hb]	0.924	0.683	0.934	0.315
SOD [U/g Hb]	0.940	0.391	0.952	0.571

Table 5. Multivariate analysis of variance for repeated measures.

Parameter	Effect	F	(p)
CAT [U/g Hb]	Group	0.518	0.477
	Time	0.280	0.600
	Group*Time	1.494	0.231
SOD [U/g Hb]	Group	0.511	0.480
	Time	1.239	0.275
	Group*Time	0.825	0.371
GPx [U/g Hb]	Group	0.224	0.639
	Time	0.011	0.918
	Group*Time	0.681	0.416

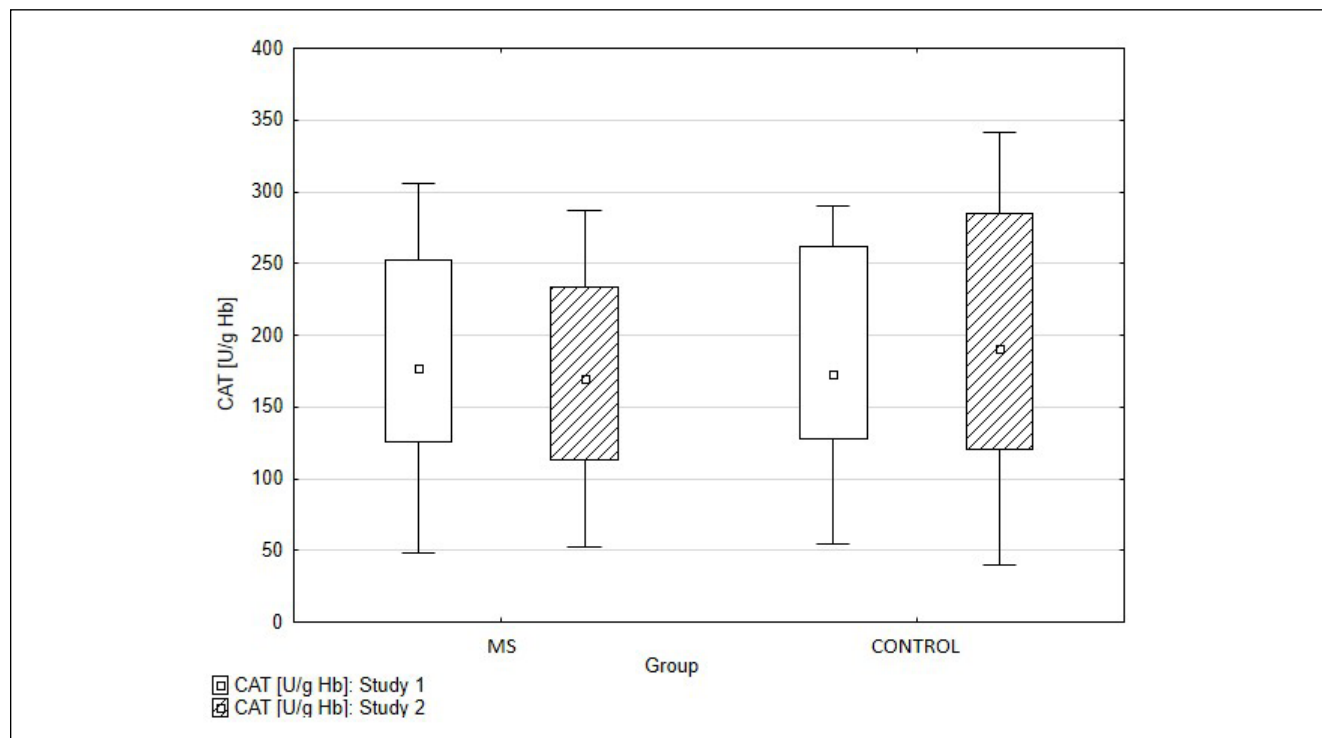


Fig. 1. CAT in the experimental group (MS) and in the control group (CONT) before (Study 1) and after (Study 2) the intervention sessions.

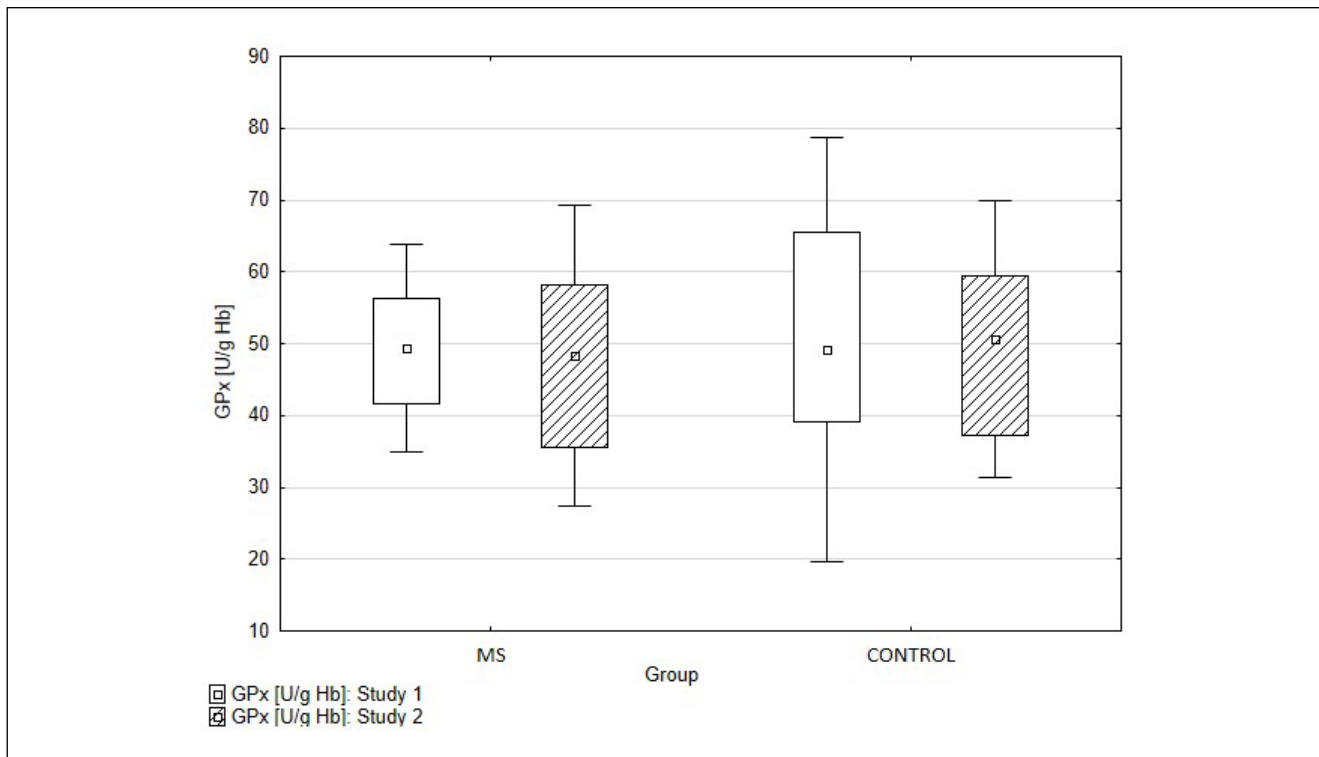


Fig. 2. GPx in the experimental group (MS) and in the control group (CONT) before (Study 1) and after (Study 2) the intervention sessions.

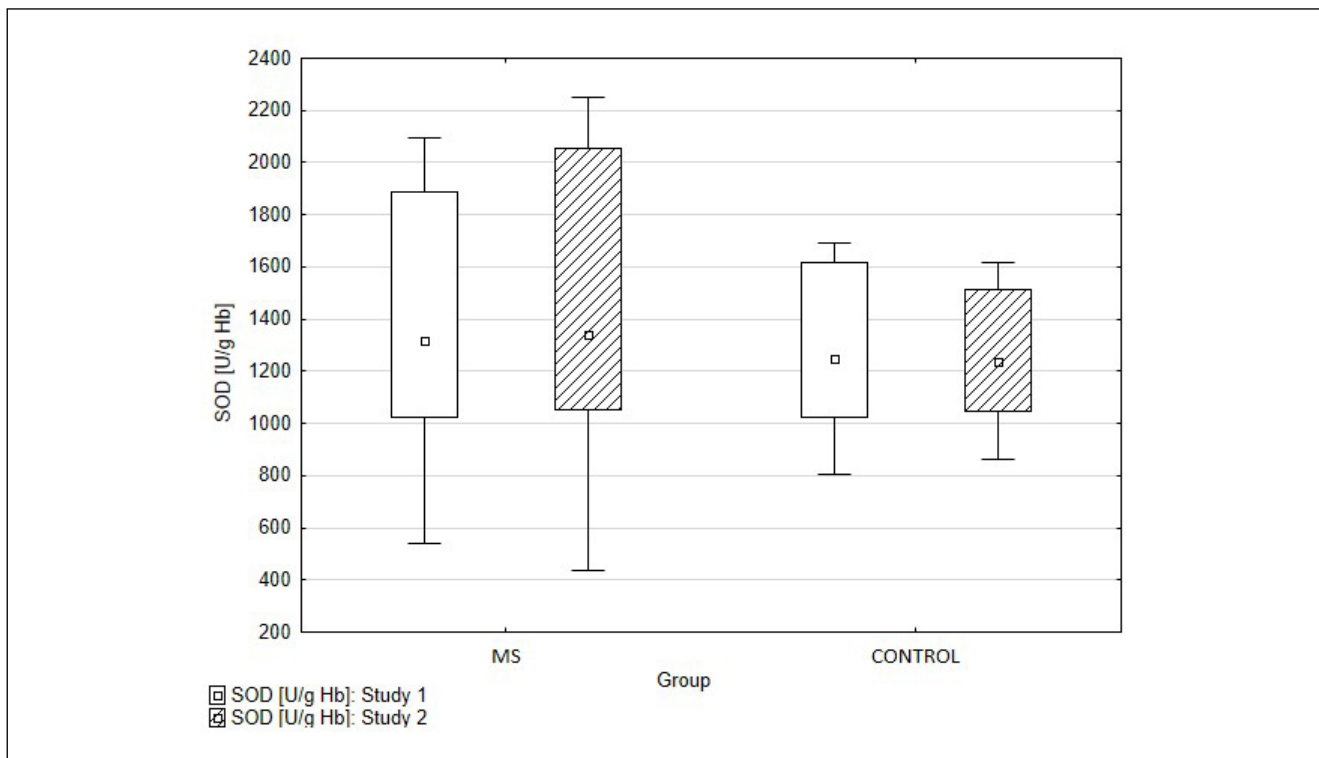


Fig. 3. SOD in the experimental group (MS) and in the control group (CONT) before (Study 1) and after (Study 2) the intervention sessions.

DISCUSSION

The present study attempted to evaluate the effects of a series of 20 WBC sessions on antioxidant enzymes (CAT, GPx, SOD) in women with MS and healthy women. Despite considerable knowledge about the pathomechanism of MS, the possibilities of pharmacotherapy are limited. For this reason, in addition to pharmacological treatment, physical activity and, above all, physiotherapy play a very important role. Oxidative stress is indicated as one of the factors involved in MS pathogenesis, especially because of the involvement of ROS in demyelinating processes. This study showed no significant changes in CAT, GPx, or SOD levels after the use of WBC. There was also no significant baseline difference between the study groups.

Oxidative stress is a neurodegenerative disorder. This is due to a long-term change in metabolism, exposure to exogenous factors and/or oxidizing compounds and is associated with an inflammatory reaction (Miller et al., 2011a; Stanek et al., 2016). Pro-oxidative-antioxidant processes play an important role in the development of several different pathologies, which can also cause adaptive changes that protect tissues against pro-antioxidant imbalances (Dugué et al., 2005). Erythrocytes may play a role in the pathophysiology of MS through altered antioxidant capacity and hemorheology, which in turn may lead to potential ischemic tissue damage (Groen et al., 2016). A study by Zagórski et al. (1991) found a decrease in SOD-1 activity in red blood cells of MS patients compared to healthy controls, which may suggest a weakening of endogenous enzymatic protective mechanisms against oxidative stress. Tasset et al. (2012), in turn, observed higher SOD values and lower GPx in patients with relapsing-remitting MS (RR-MS) than in healthy controls. Gray et al. (2014) also showed that microglia catalase activity is increased in gray matter MS and is an important endogenous antioxidant in MS. In several studies, data can be found in healthy and/or training people, as well as with a different number of WBC treatments. Lubkowska et al. (2009) observed in 10 healthy men that a single WBC treatment (-130°C) caused an immediate increase in the activity of GPx and glutathione reductase (R-GSSG) and a decrease in the activity of CAT and glutathione transferase (T-GSH). In the next study, the authors correlated changes in antioxidant enzyme activity in healthy men with the number of WBC treatments. The activity of individual antioxidant enzymes depended on the exposure time. Ten WBC treatments resulted in slight changes in the activity of SOD, GPx, and glutathione reductase (GR) with a downward trend and a marked increase in CAT activity and glutathione lev-

el. After 20 WBC treatments, CAT activity returned to baseline, but SOD activity increased, and GPx activity decreased further. The authors came to a very important conclusion that WBC increased oxidative stress and caused the accompanying decrease in the activity of antioxidant enzymes after ten sessions, with another compensatory surge after the end of the 20-session cycle (Lubkowska et al., 2012). The results of the study by Mila-Kierzenkowska et al. (2013) suggest that even a one-time WBC before exercise in volleyball players may have a beneficial effect on the body's antioxidant system and alleviate the symptoms of oxidative stress caused by exercise. Stanek et al. (2016) studied 32 healthy men: 16 men using WBC and kinesiotherapy and 16 men only exercising. They did not notice any significant changes in the activity of antioxidant enzymes (SOD, SOD-Mn, SOD-ZnCu, CAT, GPx, GR) in the case of WBC alone; however, other indicators (TAS, TOS, malondialdehyde – MDA, oxidative stress index – OSI) confirmed that WBC reduced oxidative stress in healthy men. In another study, the same authors also observed that WBC treatments had a beneficial effect on endothelial parameters (Stanek et al., 2023). Wojciak et al. (2020) showed that a series of WBC can be a method to increase, among other things, the antioxidant defense in men; however, this effect depended on many factors: the number of treatments, age, and level of physical activity. In our study, we did not observe statistically significant changes in the activity of CAT, GPx, or SOD in healthy people after the use of WBC.

There is also data available on the effects of WBC on oxidative stress in people with MS. Miller et al. (2010) investigated the effect of WBC on plasma oxidative stress (TAS) and the activity of antioxidant enzymes (CAT, SOD). Patients participated in 30 WBC sessions, of which the last ten sessions were additionally combined with a 14-day melatonin supplementation (10 mg per day). Researchers saw an increase in TAS but no changes in SOD and CAT. Supplementation of melatonin and WBC together significantly increased the activity of SOD and CAT in the red blood cells of MS patients. Researchers also observed that antioxidant enzymes (SOD) enzymes were initially reduced in MS patients. The aim of the next study by Miller et al. (2011a) was to compare the effect of WBC on the total antioxidant status (TAS) of plasma and the activity of selected antioxidant enzymes in erythrocytes (SOD and CAT) in patients with depressive and non-depressive MS. The patients underwent ten exposures in a cryochamber. WBC caused a significant increase in TAS levels but showed no effect on the activity of SOD and CAT. In another study, Miller et al. (2011b) determined the effect of 10 WBCs supplemented with 10 mg of melatonin (in some patients – 16/28) on TAS, SOD and CAT in MS patients

(-120°C/3 min/day). In patients with additional supplementation, a statistically significant increase in the activity of SOD and CAT was observed. The aim of the study by Bryczkowska et al. (2018) was to assess the effect of 30 WBCs (3 min at -130°C) on the basic biochemical parameters of blood and the activity of major antioxidant enzymes in red blood cells of MS patients. Researchers observed a significant increase in only SOD1 after the treatments. WBC appears to have improved the body's antioxidant capacity in MS patients, although our studies did not detect changes in the activity of antioxidant enzymes in RBCs. More research is needed to elucidate the antioxidant mechanisms and determine the exact effects of using a cryochamber.

CONCLUSIONS

There were no changes in the examined parameters (CAT, GPx, SOD) after the use of WBC in the group with MS and in the control group. There were also no differences between the groups in either the first or the last study. There were also no adverse changes in the parameters tested – WBC seems to be a safe form of therapy, although the mechanisms of hypothermic protection are not fully understood. Research into the various forms of therapy that affect the antioxidant capacity of MS patients should continue.

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