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Acute effect of a single whole-body cryostimulation on prooxidant–antioxidant balance in blood of healthy, young men

Anna Lubkowska [a,*], Monika Chudecka [b], Andrzej Klimek [c], Zbigniew Szyguła [d], Barbara Frączek [e]

[a] Department of Physiology, Faculty of Natural Sciences of Szczecin University, al. Piastów 40b, 71-065 Szczecin, Poland
[b] Department of Anthropology, Faculty of Natural Sciences of Szczecin University, al. Piastów 40b, 71-065 Szczecin, Poland
[c] Institute of Human Physiology, University School of Physical Education, P.O. Box 62 (AWF), 31-571 Krakow, Poland
[d] Department of Sports Medicine, University School of Physical Education, P.O. Box 62 (AWF), 31-571 Krakow, Poland
[e] Department of Human Nutrition, University School of Physical Education, P.O. Box 62 (AWF), 31-571 Krakow, Poland

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ABSTRACT

1. We have examined the prooxidative–antioxidative reaction to extremely low temperatures (−130 °C) during a one-time cryostimulation in 15 young, clinically healthy individuals.
2. The total lipid peroxides as the total oxidative status (TOS) and the total antioxidative status (TAS) were measured in blood plasma collected in the morning of the day of cryostimulation, 30 min after the cryostimulation, and on the following morning.
3. The level of stress expressed by total oxidative status in plasma, resulting from exposure to extremely low temperatures, was statistically significantly lowered 30 min after leaving the cryochamber than prior to the exposure. The next day, the TOS level still remained lower than the initial values. The TAS level decreased after leaving the cryochamber and remained elevated the following day.

1. Introduction

The whole-body cryotherapy (or cryostimulation) has found its application in treating many diseases. In professional sports, its usage has been beneficial in improving biological regeneration and recovery from post-exercise muscle injury (Banfi et al., 2008; Zimmer, 2003). Local or systemic use of extremely low temperature speeds up the healing process of impaired tissues, weakens inflammable reaction, lowers muscle spasticity, and also has analgesic properties (Yaumauchi et al., 1981; Bauer and Skrzek, 1999; Hubbard et al., 2004; Nadler et al., 2004; Krasuski and Dederko, 2005).

Cooling tissue causes a decrease in the efficiency of cellular respiration, releases enzymes from impaired cells, and inhibits the breakdown of high-energy compounds (ATP, CP) and glycogen (Zimmer, 2003). Cryostimulation causes muscle trembling and a reduction in metabolism by about 50%, which decreases oxygen demand. After 4 min of exposure to extremely low temperature (from −100 to −130 °C), the body experiences considerable haemangiectasia (angio-osteodystrophy) and an increase in blood supply to internal organs, which leads to an increase in muscle oxygen concentration (Bauer and Skrzek, 1999; Zagrobelny and Zimmer, 1999). A subsequent effect of exposing the body to cryogenic temperature is a fall in lactate and histamine concentration levels and an increase in bradykinin and angiotensin concentrations, which, in effect, cause a considerable pain reduction (Griffin and Reddin, 1981). Cryostimulation has also been noted to increase the secretion of adrenotropic hormones (ACTH), cortisol, adrenaline, norepinephrine, and testosterone in plasma. Stimulation of adrenocorticotropin has been known to reduce inflammation processes (Leppäluoto et al., 2008). Initially, cryotherapy was predominantly used on protracted inflammable states accompanying rheumatic diseases. Research on the influence of cryostimulation on the production of reactive oxygen species (ROS), lipid peroxidation and the antioxidative response of the body, focused mostly on the treatment of people with rheumatoid arthritis (Yaumauchi et al., 1981; Janiszewski, 1998; Księżopolska-Pietrzak et al., 1999; Metzger et al., 2000). The cryotherapeutic treatment of these individuals was accompanied by kinesitherapy as an integral component of this form of rehabilitation, and in the case of athletes, cryostimulation was accompanied by physical training (Swenson et al., 1996; Woźniak

* Corresponding author. Tel.: +48 91 444 2754.
E-mail address: annalubkowska@tlen.pl (A. Lubkowska).

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et al., 2002, 2007b; Krasuski and Dederko, 2005). In both cases, it is difficult to interpret clearly the influence of low temperature due to an increase in lipid peroxidation that is a result of an increased ROS production during physical effort, as confirmed by numerous studies (Alessio, 1993; Urso and Clarkson, 2003; Bloomer et al., 2005, 2006; Metin et al., 2003).

An exposure to an acute cold temperature represents an obvious stress, which could lead to various physiological and metabolic reactions in the organism. Among others, prooxidant–antioxidant processes play an important role in the development of several various pathologies that could also trigger adaptation changes to protect tissues against disturbances in the pro–antioxidant balance (Dugué et al., 2005; Wozniak et al., 2007b). Limited studies on the effect of whole-body cryostimulation on total oxidative status (TOS) and total antioxidative status (TAS) in the plasma of healthy men not involved in physical exercise exist. Thus, the aim of this study was to assess pro–antioxidant status in healthy, young men after one session of whole-body cryostimulation.

2. Materials and methods

Fifteen healthy, ±21 year old men, with a normal body weight (body mass index BMI < 28) and never previously exposed to any form of cryotherapy, were recruited for this study.

The characteristics of the examined group are presented in Table 1. All participants were asked to sign a written consent. The project of the study was approved by the Bioethical Committee of the Regional Medical Society in Cracow. Prior to engaging in the experiment, all participants underwent a physical examination to exclude any contraindications against cryostimulation. The individuals were exposed to a one-time session of extremely low temperature (−130 °C) in a cryogenic chamber at the Małopolska Center of Cryotherapy in Cracow, Poland, in groups of 4 persons each. The session lasted 3 min.

Each participant's entry to the cryochamber was preceded by a 30 s adaptation in the vestibule at a temperature of −60 °C. During the procedure, the participants wore shorts, socks, wooden clogs, gloves and a hat covering the auricles against frostbite. Blood samples were obtained from an antecubital forearm vein after a 10 min rest in a sitting position, using vacutainer system tubes (Sarstedt, Germany). During the day of cryostimulation, blood specimens were collected after an overnight fasting, in the morning between 8.00 and 8.30 am (sample A), 30 min after cryostimulation, e.g. 9.00–9.30 am (sample B), and the next morning, between 8.00 and 8:30 am (sample C). We examined the entire blood morphology and carried out the smear analysis. After centrifugation, the serum and plasma were divided into aliquots and immediately deep-frozen at −70 °C. The total lipid peroxides as the total oxidative status (TOS, PerOx) and the total antioxidative status (TAS, ImAnOx) were measured with the photometric test method, Immunodiagnostik AG, Bensheim-Germany. The sensitivity of the assay for TOS was 7 μmol/L, and the intra- and inter-assay variability were ⩽3.1% and ⩽5.1%, respectively. Detection limit for the TAS kit was 130 μmol/L, and the intra- and inter-assay variability were ⩽2.3% and ⩽2.43%, respectively.

2.1. Statistics

Statistical analysis was performed using the Statistica 6 package. Data were checked for normal distribution using the Shapiro–Wilk test. Since in some cases the data distribution was not normal, the Friedman's ANOVA for repeated measurements was applied to examine the overall changes. Thereafter, the Wilcoxon signed-rank test for paired non-parametric data was used to determine variations from the initial levels during the experiment, as recommended for this type of data (Cohen, 1988). Values of $p < 0.05$ were considered statistically significant. In addition, correlation coefficients between variables were calculated using a Spearman analysis.

3. Results

The morphological parameters and haematologic coefficients were observed to be within clinical and laboratory norms in all the examined individuals (Table 1). The results of responses to one-time whole-body cryostimulation are presented in Figs. 1 and 2.

According to the values provided by Immunodiagnostik AG, we found that the level of stress, expressed by means of the total oxidative stress (TOS = PerOx) in plasma was very low = 138 μmol/L (85–160 μmol/L) in the initial samples (A). As a result of cryostimulation we observed a statistically significant decrease in TOS, to 131.1 μmol/L (64–165 μmol/L) 30 min after leaving the cryochamber (B). On the day following the cryostimulation, the TOS level rose slightly, to 132 μmol/L (87–143 μmol/L) (C) and was

Table 1
Physical characteristics, hematological parameters and cortisol levels in the serum of the examined men (the values are mean ± SD, minimum, and maximum)

	n	Mean value ± standard deviation (SD)	Min	Max
Height (cm)	15	180.15 ± 7.59	166.00	190.00
Body mass (kg)		74.70 ± 6.52	63.00	86.50
BMI (kg/m^2)		23.04 ± 1.65	21.05	25.40
WBC (10^3/μL)		4.36 ± 1.31	1.51	5.54
RBC (10^6/μL)		5.19 ± 0.10	5.09	5.41
HGB (g/dL)		15.23 ± 0.53	14.5	16.2
HCT (%)		45.55 ± 1.18	43.9	47.1
MCV (fL)		87.82 ± 2.06	85.2	90.4
MCH (pg)		29.39 ± 0.83	28.1	31.0
PLT (10^3/μL)		230.73 ± 38.53	139.0	287.0
Cortisol A (μg/dl)		10.63 ± 3.16	5.78	16.1
Cortisol B (μg/dl)		10.10 ± 2.77	6.26	15.2

BMI—body mass index; WBC—white body cell, RBC—red body cell, HGB—hemoglobin, HCT—hematocrit, MCV—mean corpuscular volume, MCH—mean corpuscular hemoglobin, MCHC—mean corpuscular hemoglobin concentration, PLT—platelets, Cortisol A—serum cortisol values before, and Cortisol B—30 min after cryostimulation.

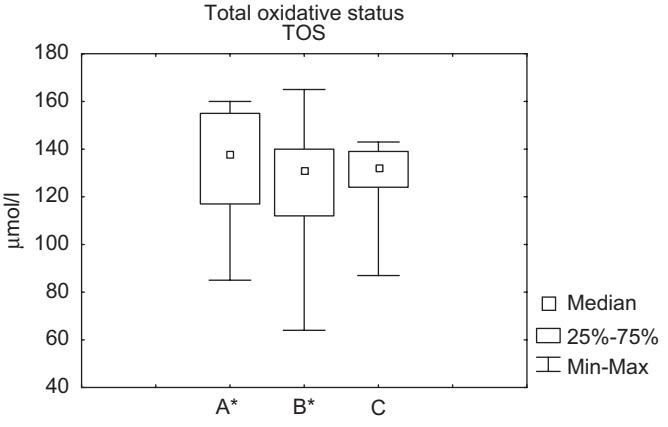

Fig. 1. Changes in plasma total oxidative status in healthy subjects at rest (A), at 30 min after cryostimulation (B) and in the morning the day after (C). *$p \leqslant 0.05$ statistically significant difference B vs. A.

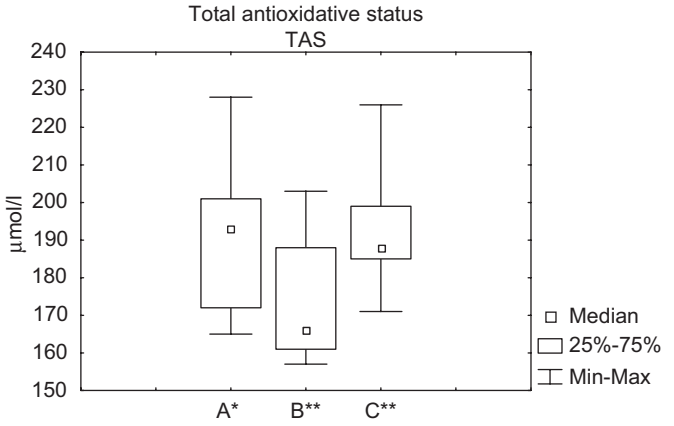

Fig. 2. Changes in plasma total antioxidative status in healthy subjects at rest (A), at 30 min after cryostimulation (B), and in the morning the day after (C). $^{*}p \leqslant 0.05$ statistically significant difference B vs. A, $^{**}p \leqslant 0.01$ statistically significant difference B vs. C.

still lower than the initial values, although the difference was not statistically significant. Similarly, the initial level (A) of the plasma total antioxidative status (TAS = ImAnOx): 193.00 µmol/L (165–228 µmol/L) was low. The TAS level significantly decreased to 166 µmol/L (157–203 µmol/L) 30 min after leaving the cryochamber (B). The following day (C), an increase in TAS level up to 188 µmol/L (171–226 µmol/L) was observed, and was statistically significant in relation to the B measurement the day before. One-time cryostimulation did not lead to significant changes in serum cortisol levels (Table 1).

4. Discussion

The reaction of the human body to extremely low temperature in a cryochamber initially resulted in a vasospasm and then vasodilation and massive tissue hyperemia (Janiszewski, 1998; Zagrobelny and Zimmer, 1999; Nadler et al., 2004). As a result of a reaction catalyzed by xanthine oxidase due to reperfusion, an increase of ROS, causing damage to both nucleic acids and proteins as well as lipid peroxidation was observed. Due to thermogenic trembling, the production of heat can exceed 3–5 times the level of the heat produced in normal metabolic state (Jackson and Sammut, 2004). Simultaneously, heat is lost due to convection and the demand for ATP increases. A higher demand for ATP increases the metabolism of oxygen in the mitochondria (Księżopolska-Pietrzak et al., 1999; Bartosz, 2003). This leads to the intensification of ROS generation, through a one-electron reduction of oxygen (Kopprasch et al., 1997). Due to the cooling and stimulation of metabolism, the mitochondria in a body exposed to extremely low temperature conditions produce 10 times more anion-radical superoxides (and after its dismutation, perhydride) (Bartosz, 2003). Additionally, in hepatocyte peroxisomes an intensification of the β-oxidation of fatty acids and the consequential generation of perhydride occur (Hamel et al., 2001).

Prior to carrying out the experiment, we anticipated that an increase in the concentration of lipid peroxidation products would be seen, which was to increase plasma total oxidative level in the subsequent blood samples after a single stay session in the cryogenic chamber. However, our results show a statistically significant drop in TOS, accompanied by a decrease in TAS in plasma. Both parameters correlated highly with each other in the B samples collected 30 min after leaving the cryochamber. It is likely to be a result of a decreased ROS generation or a significant participation of non-enzymatic systems in their removal. Zagrobelny et al. (1993) observed a significant increase in the concentration of adrenaline, norepinephrine, ACTH, and beta-endorphins in blood serum 30 min after cryostimulation. Catecholamines—important regulators of metabolism—may have some impact on the production of reactive oxygen species.

Whilst only few studies on the influence of cryostimulation on oxidative and antioxidative processes in the cells of ill and healthy individuals have been published, it is suggested that repeated cryochamber sessions may cause adaptive changes, for example an increase in antioxidative capacity (Janiszewski, 1998). Woźniak et al. (2007b) when compared with the activity of superoxide dismutase, catalase, and glutathione peroxidase after training accompanied by cryostimulation. It was found that their activities were lower when training was preceded by exposure to extremely low temperature conditions. Additionally, in the next experiment it was found that low temperature solely caused neither a labialisation of lysosomal membranes nor significant changes in the activities of lysosomal hydrolases (Woźniak et al., 2007a). Siems and Brenke (1992) and Siems et al. (1994) observed that acute cold stimuli (such as winter swimming) induced a decrease in major plasma antioxidants (i.e. ascorbic acid and uric acid) and an increase in the concentration of hydroxynonenal in plasma (a marker of lipid peroxidation).

Dugué et al. (2005) compared acute and long-term changes in plasma antioxidant capacity in women who attended whole-body cryotherapy regularly. They observed a significant increase in the value of total peroxyl radical trapping antioxidant capacity of plasma (TRAP) 2 min after cold stress in the first 4 weeks of their study. Thirty-five minutes after application of cold stress, the values of TRAP did not vary from the baseline values. These data may suggest that cold stress activates antioxidant defense in the body, especially at the initial stages of an adaptation period. However, changes in the TRAP values showed significant variations between subjects. High individual variation of response to stress caused by cold is confirmed by our own research, in particular with regard to total oxidative status, which enables estimation of lipid peroxidation increase.

Cortisol, the concentration of which increases in response to stressogenic factors, is generally the most frequently investigated marker in cryostimulation research. Reports on changes in its concentrations are often contradictory. In this research, we did not note any changes in cortisol concentration in serum caused by a one-time session of cryotherapy. Zagrobelny and Zimmer (1999) reported an increase in the concentration of this hormone in blood serum after cryotherapy treatment, but Woźniak et al. (2007a) did not report any statistically significant changes in cortisol concentrations both after a single stay session in a cryochamber, and after 6 days of training accompanied by cryostimulation. They observed, however, an increasing tendency in cortisol levels after the sixth day of training, and then a decrease after the 10th day of training in conjunction with cryostimulation. Leppäluoto et al. (2008) studying the effects of long-term winter swimming and whole-body cryotherapy, observed that plasma cortisol exhibited an insignificant increase after the first winter swimming, as did ACTH. During whole-body cryotherapy, plasma cortisol at 15 min after exposure was significantly lower in week 4 than in week 1. After the 11th week of the study, plasma cortisol levels were lower at 15 and 35 min in comparison with the period preceding the start of the experiment.

Limited literature on the influence of cryogenic temperatures on antioxidative mechanism and on the generation of free radicals leaves room for future research. The next step should be to estimate the influence of cryostimulation series (in arrangements most often used by patients and athletes) on plasma oxidative–antioxidative coefficients.

In conclusion, one session of whole-body cryostimulation causes disturbances in the prooxidant–antioxidant balance—the level of total oxidative status in plasma was statistically significantly lowered 30 min after leaving cryochamber and remained low the following day, whereas the level of total antioxidative status decreased after cold exposure and elevated the next day.

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