IMPACT OF 10 SESSIONS OF WHOLE BODY CRYOSTIMULATION ON AEROBIC AND ANAEROBIC CAPACITY AND ON SELECTED BLOOD COUNT PARAMETERS

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ABSTRACT: The systemic effect of low temperature suggests that sessions in a cryogenic chamber might improve athletes' capacity as a standard element of training. Therefore the authors decided to evaluate the impact of 10 sessions of whole body cryostimulation (WBCT) on aerobic and anaerobic efficiency as well as on selected blood count parameters. The study group included 32 volunteers – 16 women and 16 men. The volunteers underwent 10 sessions of WBCT in a cryogenic chamber. Blood samples (RBC, WBC, PLT, HGB, HCT) were taken, and aerobic and anaerobic efficiency and lactate concentration in capillary blood were measured before the first session and one day after the last one. No significant differences were observed in values of aerobic capacity after 10 sessions of WBCT. There was a rising trend in men and a declining trend in women. The lactate concentration did not differ significantly before and after WBCT. A slight rise in aerobic and anaerobic threshold was observed in men, while in women the values slightly fell. The Wingate test showed no significant differences in results before and after cryostimulation. Only the TOBT was significantly shorter in men (6.12 ± 1.49 vs 3.79 ± 1.14 s). The WBCT sessions resulted in a significant rise of the haematological parameters both in women and men, excluding HCT, which showed a statistically insignificant rise. Ten sessions of whole body cryostimulation did not affect aerobic capacity in the tested group, although it improved the blood count parameters.

KEY WORDS: cryotherapy, aerobic efficiency, anaerobic efficiency

INTRODUCTION

Whole body cryostimulation (WBCT) has been applied since the 1970s, initially in Japan and then all over the world. It became especially popular in Germany, Poland, Canada, Latvia and in Scandinavian countries. The WBCT procedure is based on stimulation of the organism with extremely low temperatures (-110 to -160°C) over a very short period (1-3 minutes) to provoke vasoconstriction of the skin vessels, followed by rapid vasodilation, without reaching hypothermia. The hyperperfusion of the tissues lasts for 3 to 6 hours after the session [30], which facilitates metabolite removal, increases the capillary perfusion and improves the viability of lymphatic vessels. Other changes have also been observed, such as decrease of sedimentation rate and other inflammatory markers (C-reactive protein, seromucoid, immunoglobulins G and A) [24,25], and altered concentrations of some hormones (increased level of ACTH, adrenaline, noradrenaline, cortisol, testosterone in men and β -endorphins) [13,15]. There are controversies regarding improved blood count parameters. Banfi et al. [3] and Klimek et al. [12] did not note any improvement, while Stanek et al. [24] observed a significant rise in the number of erythrocytes (RBC), haemoglobin concentration (HGB)

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and mean erythrocyte volume (MCV). Cryogenic temperatures also lead to a marked decrease of peripheral receptors' reactivity and slow down the neural conductivity (by $15 \text{ m} \cdot \text{s}^{-1}$ with local temperature decline by 10° C) [4]. Physiological reaction of the organism to low temperature results in the following effects: analgesic [15,16], anti inflammatory [2], anti-oedematous [6], antidepressant [22], relaxing (decreased muscular tonus) [11], and antioxidant [1,8,29]. Complex effects of cryotherapy combined with lack of side effects resulted in its wide application in medicine – mainly in locomotor system therapy, sports medicine and in regeneration procedures [18,19].

The systemic effect of low temperature suggests that sessions in a cryogenic chamber might improve athletes' capacity as a standard element of training. During the reactive hyperaemia after WBCT, the supply of oxygen and energetic substrates (mainly glucose) to the cells improves. At the same time, faster removal of the metabolites (especially lactate) maintains homeostasis of the organism and proper pH, which enhances the activity of enzymes responsible for aerobic and anaerobic ATP synthesis. Increased levels of ACTH and cortisol stimulate production of free fatty acids from the triacylglycerols in the fat tissue as well as stimulating gluconeogenesis in the liver [27]. The mentioned effects increase bioenergetic capability of the cell and can directly influence the organism's efficiency. Therefore the authors decided to evaluate the impact of 10 sessions of WBCT on aerobic and anaerobic efficiency as well as on selected blood count parameters.

MATERIALS AND METHODS

The study group included 32 volunteers – 16 women and 16 men, students of the Faculty for Physical Education and Physiotherapy of Opole Technical University, who had never had cryotherapy before. Prior to the test they had a medical examination to rule out contraindications for cryonic sessions and maximal load efforts.

Prior to the test, basic anthropometric measurements were performed. Body weight and composition were evaluated using an electric impedance meter (Tanita Body Composition Analyzer, TBF-330). The results are shown in Table 1.

The volunteers underwent 10 sessions of WBCT in a cryogenic chamber of Pulmonology-Rheumatology Hospital Trust in Pokój (Poland). The procedures took place once daily in the morning from Monday till Friday, for 3 minutes at a temperature of -130°C. Each session was preceded by adaptation at a temperature of -60°C for 30 seconds. Participants' dressing was consistent with regulation for cryonic procedures (swimsuits, covered feet, hands, ears and airways). Blood samples were taken before the first session and one day after

TABLE I. SOMATIC CHARACTERS OF FEMALE AND MALE STUDYPARTICIPANTS

Variables	Male [n=16]	Female [n=16]
AGE [years]	21.69±1.89	20.13±0.95
HIGHT [cm]	190.56±5.20	162.46±6.41*
BODY MASS [kg]	84.87±8.74	53.65±7.94*
BODY MASS INDEX [kg · cm ⁻²]	23.38±2.31	20.21±1.98*
FAT [%]	15.04±10.45	19.81±2.26*
FAT MASS [kg]	11.26±4.14	10.83±2.57
FAT-FREE MASS [kg]	73.60±5.24	42.79±5.73*

Note: The values are mean ± standard deviation.

* Statistically significant differences between men and women

the last one to evaluate RBC, HGB, haematocrit (HCT), leucocyte count (WBC) and platelet count (PLT).

Measurements of aerobic and anaerobic efficiency were performed before and after the series of WBCT. Aerobic efficiency was evaluated with the test of gradually increasing intensity using the cycloergometer (Excalibur Sport Lode, Holland). The initial load was 1 W per kg of fat-free body mass (1W·kgFFM⁻¹), increased by 0.5W·kgFFM⁻¹) until participant's refusal. Respiratory parameters were measured using a gas analyser (Quark b2, Cosmed, Italy). Heart rate was measured with a sport tester (Polar S610i, Finland). The following parameters were recorded continuously: oxygen uptake (VO2), expired carbon dioxide ($\dot{V}CO_2$), fraction of oxygen in expired air (FEO₂), fraction of carbon dioxide in expired air (FECO₂), minute ventilation (VE), tidal volume (TV), respiration frequency (RF), ventilator equivalent ratio for oxygen (VE $\cdot \dot{VO}_2^{-1}$), ventilator equivalent ratio for carbon dioxide (VE · VCO2-1). These values allowed calculation of the ventilatory aerobic (AT) and anaerobic (ANT) thresholds for each participant. To determine AT, the maximum value of FEO₂, significant increase in VE and the minimum value of VE $\cdot \dot{VO}_2^{-1}$ were used, while maximum value of FECO₂, significant increase in VE, and the minimum value of VE \cdot VCO₂⁻¹ were used for the determination of ANT [20].

Anaerobic capacity was evaluated using the 20-s Wingate test. According to Laurent et al. [14], when compared to the 30-s test, the 20-s one may be considered a valid alternative to measure the final power output values. The Wingate test was performed using the cycloergometer Ergomedic 894E (Monark, Sweden). The test was preceded by 2-minute warm-up with a load of $1W \cdot 1kg^{-1}$. The test workload was 7.5% of the body mass in men and 6.5% in women. During the test, the following parameters were registered: maximal anaerobic power of lower limb (MAP), average power (AP), time to obtain and sustain MAP (T_{OBT}, T_{SUS}), fatigue index (FI), and total external work (W_{tot}).

The lactate concentration in capillary blood was measured before and after each aerobic and anaerobic efficiency test prior to and after the 10 sessions of WBCT using the lactate analyser for athletes Lactate Scout (Finland).

The normality of distribution of dependent variables was tested with the Shapiro-Wilk test. The differences between the tested

Variables	Wor	men	Men		
variables	Before WBCT	After WBCT	Before WBCT	After WBCT	
T [min]	15.92±0.82	15.64±0.91	19.82±5.95	19.46±6.38	
P _{max} [W]	185.57±24.47	177.45±23.27	348.75±42.48	341.81±53.83	
VO₂max [I∙min⁻¹]	2.37±0.26	2.27±0.29	4.31±0.49	4.48±0.65*	
VO₂max [ml⋅kg body mass⁻¹⋅min⁻¹]	45.64±2.62	43.78±3.79	51.04±5.6	54.01±6.93*	
HR _{max} [bpm]	193.13±8.72	185.43±17.28	185.81±5.75	187.44±5.62*	
La _{rest} [mmol·l ⁻¹]	1.04±0.14	0.99±0.16	0.96±0.19	1.0±0.17*	
La _{exe} [mmol·l ⁻¹]	9.09±0.66	8.74±1.03	10.84±1.8	10.93±1.65	

Note: The values are mean \pm standard deviation. T – duration of the test; P_{max} – maximal power; VO_2max - maximal oxygen uptake; HR – heart rate; LA_{rest} – resting lactate concentration; LA_{exe} – post exercise lactate concentration. * Statistically significant differences between men and women.

parameters in specific periods (dependent variables) as well as between men and women (independent variable) were evaluated with analysis of variance (ANOVA) with repeated measurements. In the case of significant effects, the differences among the medians were evaluated with the post hoc multiple comparisons test (Tukey test).

The values of the parameters are shown as mean (x) \pm standard deviation (SD). The level of statistical significance was set at p<0.05.

All participants were informed about the aim and course of the experiment and signed their written consent. The experiment was accepted by the Bioethical Committee of the Regional Medical Council in Opole (Resolution No. 163/2009).

RESULTS

No significant differences were observed in values of aerobic capacity after 10 sessions of WBCT. There was a rising trend in men and a declining trend in women (Table 2). The interaction between the groups was statistically significant (p=0.00007). The lactate concentration did not differ significantly before and after WBCT (Table 2).

A slight rise in aerobic and anaerobic threshold was observed in men, while in women the values slightly fell (Tables 3, 4). The interaction between the groups was statistically significant (p=0.00004).

The Wingate test showed no significant differences between records before and after cryostimulation. Only the T_{OBT} was significantly shorter in men (6.12±1.49 vs 3.79±1.14 s) and in women it remained stable (Table 5). The interaction between the groups was statistically significant (p=0.003).

The WBCT sessions resulted in a significant rise of the haematological parameters both in women and men, excluding HCT, which showed a statistically insignificant rise (Table 6).

DISCUSSION

Whole body cryotherapy is more and more frequently applied in athletes as a method of choice in the case of locomotor system injuries or as an element of regeneration. Evidence for its beneficial impact on sport efficiency is being researched, but there are very few, often contradictory, reports on this topic. Hagner et al. [10] reported an increase of some parameters measured in the stress test according to the Bruce protocol (prolonged mean duration, increase in velocity and mean load), which should suggest improvement of aerobic efficiency parameters. Uckert et al. [28] observed increased running endurance after cooling with an ice vest about 20 minutes before training that was performed in high temperature. However, results obtained by Stacey et al. [23] show that immersion in cold water (10°C) does not influence capacity. Klimek et al. [12] also did not

TABLE 3. PHYSIOLOGICAL VARIABLES FOR FEMALE AND MALE PARTICIPANTS DURING THE PROGRESSIVE TEST AT THE AEROBIC THRESHOLD (AT)

) (orighted	Wor	Women		Men	
Variables	Before WBCT	After WBCT	Before WBCT	After WBCT	
T [min]	5.26±0.27	5.16±0.29	6.54±1.96	6.42±2.11	
P _{max} [W]	77.94±11.54	74.94±9.77	146.48±17.84	143.56±22.61	
∕O₂max [l∙min⁻¹]	0.99±0.11	0.95±0.12	1.81±0.21	1.88±0.27*	
√O₂max [ml⋅kg body mass⁻¹⋅min⁻¹]	19.17±1.1	18.26±1.57	21.44±2.36	22.68±2.91*	
HR _{max} [bpm]	135.19±6.1	128.95±12.12	130.07±4.03	130.21±3.93*	
% V̇O₂max	35.52±2.1	34.79±3.0	40.83±4.49	43.21±5.54*	
% HR _{max}	67.59±3.05	64.48±6.06	65.03±2.01	65.6±1.97*	

Note: The values are mean \pm standard deviation. T – time to reach AT; P_{max} – maximal power; VO₂max - maximal oxygen uptake; HR – heart rate. * Statistically significant differences between men and women.

TABLE 4. PHYSIOLOGICAL	VARIABLES FOR FEMALE	AND MALE PARTICIP	ANTS DURING THE	PROGRESSIVE TEST	AT THE ANAEROBIC
THRESHOLD (ANT)					

Variables	Women		Men	
variables	Before WBCT	After WBCT	Before WBCT	After WBCT
T [min]	9.57±0.49	9.38±0.54	11.89±3.57	11.67±3.83
P _{max} [W]	124.33±18.41	118.89±15.59	233.66±28.46	229.01±36.06
VO₂max [I · min⁻¹]	1.84±0.19	1.76±0.22	2.89±0.33	3.07±0.51*
VO₂max [ml⋅kg body mass⁻¹⋅min⁻¹]	30.58±1.76	29.14±2.51	34.19±3.76	36.19±4.64*
HR _{max} [bpm]	154.5±6.97	147.37±13.85	148.65±4.6	149.95±4.96*
% VO₂max	54.77±3.15	52.18±4.5	61.25±6.73	64.81±8.31*
% HR _{max}	80.3±2.33	79.49±1.97	83.11±2.81	82.27±2.16

Note: The values are mean \pm standard deviation. T – time to reach AT; P_{max} – maximal power; \dot{VO}_2 max - maximal oxygen uptake; HR – heart rate. * Statistically significant differences between men and women.

TABLE 5. VARIABLES CHARACTERIZING ANAEROBIC POWER AND CAPACITY FOR FEMALE AND MALE PARTICIPANTS

	Wo	men	Men	
Variables	Before WBCT	After WBCT	Before WBCT	After WBCT
MAP [W]	461.31±97.97	490.96±109.01	924.76±119.84	914.0±138.17*
AP [W]	391.7±74.62	415.49±86.18	768.19±88.17	744.01±127.93*
FI [W⋅kg body mass⁻¹⋅s⁻¹]	0.26±0.09	0.24±0.07	0.29±0.08	0.29±0.06
tobt [s]	8.47±2.48	8.4±2.17	6.12±1.49	3.79±1.14 [†] *
Tsus [s]	3.27±1.35	3.84±1.65	3.38±0.75	3.11±1.12
La _{rest} [mmol·l⁻¹]	0.93±0.1	0.93±0.12	0.88±0.12	0.93±0.15
La _{exe} [mmol·l⁻¹]	10.05±1.35	10.42±1.21	10.11±0.85	10.79±1.05

Note: The values are mean \pm standard deviation. MAP – maximal anaerobic power of lower limb; AP – average power; FI – fatigue index; T_{OBT} – time to obtain MAP; T_{SUS} – time to sustain MAP; LA_{rest} – resting lactate concentration; LA_{exe} – post-exercise lactate concentration. [†] Statistically significant difference: before and after 10 sessions of WBCT. * Statistically significant differences between men and women.

TABLE 6. SELECTED MORPHOLOGICAL BLOOD VARIABLES FOR FEMALE AND MALE PARTIC	IPANTS

	Wor	Women		Men	
Variables	Before WBCT	After WBCT	Before WBCT	After WBCT	
RBC [10 ⁶ ·µl ⁻¹]	4.54±0.22	4.84±0.29 [†]	5.22±0.85	5.56±0.44 [†]	
HGB [g·dl⁻¹]	13.54±0.51	14.44±0.68 [†]	15.81±0.58	16.5±0.68 [†]	
HCT [%]	37.93±1.01	39.07±1.13	45.23±2.11	46.82±1.52*	
WBC	51.59±1.83	55.48±2.47 [†]	46.84±1.68	50.35±2.35 [†]	
PLT [10 ³ ·µl⁻¹]	247.89±25.12	301.39±43.23 [†]	264±27.53	320.06±42.84 [†]	

Note: The values are mean ± standard deviation. RBC – red blood cells; HGB - haemoglobin concentration; HCT – haematocrite; WBC – white blood cells; PLT – platelets. [†] Statistically significant difference: before and after 10 sessions of WBCT. * Statistically significant differences between men and women.

observe increased aerobic efficiency after WBCT. In the authors' own study, the aerobic efficiency as well as lactate concentration before and after 10 sessions of WBCT did not alter significantly, although an increasing trend in capacity parameters (maximal oxygen absorption, minute ventilation, shift of aerobic and anaerobic thresholds to the right) was observed in men. Maybe a larger number of sessions would result in visible, statistically significant changes in efficiency. Hagner et al. [10] reported improved aerobic efficiency after 20 sessions, whereas Klimek et al. [12] reported no improvement after 10 WBCT sessions. The issue of optimal number of sessions warrants further studies. Increase of the parameters can be explained by increase of erythrocyte number and haemoglobin concentration after stimulation with cold temperatures. Similar results regarding response of haematological parameters to cold were observed by Stanek et al. [24]. On the other hand, Klimek et al. [12], Biały et al. [5] and Banfi et al. [3] observed stable or decreasing values of those parameters. A significant rise in RBC and HGB after 10 sessions of WBCT was observed by the authors in women, which, similarly to the male group, suggested an increase of aerobic efficiency parameters. However, the authors observed decreased values of these parameters in women, which may suggest some metabolic changes at the cell level. It was shown that in mitochondria oestrogens inhibit I, II, III and IV complexes of the respiratory chain and ATP synthesis. Oestrogens specifically inhibit the activity of the proteins of the respiratory chain (probably due to phosphorylation) and can modify the results of the aerobic efficiency tests [9]. Progesterone can also influence cell respiration in mitochondria. It was proved that it can facilitate mitochondrial respiration [21]. These facts should suggest the dependence of aerobic energetic processes on sex hormones in women. In the period of 14 days between the measurements, the levels of oestrogens and progesterone had surely changed, altering the results.

It is supposed that low temperatures stimulate leukocyte production, which improves the organism's immunity [11,12,23]. The present authors' research supports this thesis, showing a significantly higher number of leukocytes after 10 sessions of WBCT. A significantly increased number of platelets was also observed, suggesting that cryostimulation may mobilize bone marrow to produce more morphotic elements both in women and men. Research done by Biały et al. [5] supports this thesis, showing a markedly increased number of reticulocytes after cryogenic procedures. The bone marrow response may result from hormonal stimulation. Secretion of ACTH and cortisol increases at low temperatures [13,15], which may result in more efficient function of the red bone marrow. However, Banfi et al. [2] suppose that cryostimulation has no impact on bone marrow function. All authors are consistent that alterations of the number of morphotic elements due to low temperatures do not exceed normal values [2,5,11,13,15].

Klimek et al. [12] and Duffield et al. [7] reported a beneficial impact of low temperature on anaerobic efficiency, although authors' own results did not show any significant differences in parameters of anaerobic efficiency before and after 10 sessions of WBCT, in men

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or women. Only the time to reach maximal power was significantly shortened in men, resulting probably from decreased maximal power measured in the Wingate test. It is difficult to interpret and compare the results of the previous studies because of varying methodology. The impact of cryogenic procedures on the human organism and its function warrants further studies.

CONCLUSIONS

Ten sessions of whole body cryostimulation did not affect aerobic and anaerobic capacity in the tested group, although it improved the blood count parameters.

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