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Thermal and hemodynamic response to whole-body cryostimulation in healthy subjects ☆

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ABSTRACT

Whole-body cryotherapy (WBC) is an increasing applied cryotherapeutic method, that involves application of a cryotherapeutic factor to stimulate the body by the means of intense hypothermia of virtually the body's entire area. This method is still not well recognized in Western Europe. However in recent years it is becoming increasingly popular in sports medicine and also in clinical application.

Cryotherapeutic agents used in WBC are considered to be a strong stress stimulus which is associated with a variety of changes in functional parameters, particularly of the cardiovascular and autonomic nervous systems. However, such strong influence upon the entire body could be associated with the risk of unexpected reactions which might be dangerous for homeostasis. The present study evaluated the complex hemodynamic physiological reactions in response to WBC exposure in healthy subjects. Thirty healthy male volunteers participated. Each subject was exposed to WBC (−120°C) for 3-min. None of the participants had been exposed to such conditions previously. The research was conducted with modern and reliable measurements techniques, which assessed complex hemodynamic reactions and skin temperature changes non-invasively. All measurements were performed four times (before WBC, after WBC, WBC + 3 h and WBC + 6 h) with a Task Force Monitor (TFM – CNSystems, Medizintechnik, Gratz, Austria). Body superficial temperature was measured by infrared thermographic techniques – infra-red camera Flir P640 (Flir Systems Inc., Sweden). Our results show a significant decrease in heart rate, cardiac output, and increase in stroke volume, total peripheral resistance and baroreceptors reflex sensitivity. These changes were observed just after WBC exposure. At stages WBC + 3 h and WBC + 6 h there was observed a significant drop in baroreceptors reflex sensitivity due to increased thermogenesis. In conclusion, the present findings suggest that WBC strongly stimulates the baroreceptor cardiac reflex in response to body fluid changes which sequentially modulate HR and BP control in supine and resting healthy subjects. The study was performed on randomized and homogenic group of young healthy subjects. Our findings are important for WBC safety determination in research and clinical studies.

Introduction

The effect of thermal factors on the human body has been the subject of numerous studies over many years. Thermal stimuli are widely applied in clinical and sport practice. Thermotherapeutic stimuli, including those characterized by extremely low temperatures – cryotherapeutic factors (e.g. local and a whole-body cryotherapy – WBC), are a frequently used form of physical therapy, used commonly in Eastern Europe. Over recent years, this method has become increasingly popular in other countries [1–3]. WBC is the application of a cryotherapeutics factor (−110 °C to −160 °C) for a short time (2–3 min) to stimulate the body by the intense peripheral hypothermia of virtually its entire area. WBC utilizes the vapors of liquid gases, e.g. nitrogen or atmospheric air. Despite

Abbreviations: AR_0X, selected skin area in thermographic analysis; ANS, autonomic nervous system; BRS, baroreceptor refex sensitivity; CI, cardiac index; CO, cardiac output; dBP, diastolic blood pressure; HR, heart rate; mBP, mean blood pressure; sBP, systolic blood pressure; SI, stroke index; SV, stroke volume; TFM, Task Force Monitor; TPR, total peripheral resistance; TPRI, total peripheral resistance index; WBC, whole-body cryostimulation.

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employing thermal stimulation that utilize very low temperatures, (from −100 °C to −170 °C), when performed properly the procedure is not associated with risk of side effects, such as frostbite or other injuries to the skin [4–6]. WBC is mostly used as a procedure to relieve pain symptoms, inflammatory condition, acute injures soft tissue rheumatic disease, neurodegenaritve conditions and depressive and anxiety disorders. This method is also very popular for wellness treatment and athletic recovery [1,2,7,8].

Whole-body cryotherapy activates a number of physiological mechanisms which attempts to maintain a constant body core temperature. Acute exposure to cryotherapeutics ambient temperatures is an extreme stressful situation for a human body which induce primarily rapid and short-term regulatory mechanisms. Most of these mechanisms are strictly depended on cardiovascular functions. Constriction of peripheral blood vessels is the principal response of the body to stimulation with, cold and the resulting decrease in temperature of the involved skin area. This mechanism restricts perfusion of the skin vascular bed, and thus reduces convective and conductive thermal loss [9,10]. As a functional component of the cardiovascular system, blood pressure undergoes constant and dynamic changes. Blood pressure is modulated both by intrinsic and extrinsic factors, and the extent of these influences is partially controlled by arterial baroreceptors, particularly in terms of short-term regulation. The baroreceptors found in the aortic arch and carotid sinuses are stimulated by stretching of the walls of vessels during the rhythmic diastolic–systolic changes of arterial blood pressure. The baroreceptors play an essential role in short-term regulation but by beat-to-beat, negative-feedback regulation of blood pressure. There are evidences that in experimental animals and also human's local and a whole body cooling stimulates the hypothalamus and thus modulate the arterial pressure and heart rate response [11–13].

There are only limited reports regarding the cardiovascular and autonomic effects after WBC exposure in healthy subjects. Most available literature concerns WBC in clinical use, but there is a significant lack of randomized and objective studies focused upon healthy subjects. There are available a number of possible indications and contra-indications for WBC therapy which have been presented by members of the rehabilitation, physiotherapy, sports medicine and cryo-medicine scientific societies. Most of suggested criteria were introduced on the basis of empirical experience not of reliable scientific studies. Introducing a medical procedure without through scientific validation might not be safe for patients [9,10,14].

Thanks to available modern experimental techniques we were able to assess the cardiovascular system effectors organs non-invasively. Due to technical advances, these methods enable a reliable and reproducible measurement of many variables. Application of advanced mathematical models during non-invasive assessment allows measurements to be obtained that are comparable to those recorded using invasive methods, with a concomitant reduction in risk for examined individuals. This is particularly important in the case of the studies that involve healthy subjects examined under physiological conditions. Such individuals are usually reluctant to participate in any procedures associated with potential health risk or marked discomfort [15,16].

The aim of this study was to evaluate the complex hemodynamic physiological reactions which occur in response to WBC exposure in healthy subjects.

Material and methods

Subjects

We have studied 30 healthy male volunteers who did not meet exclusion criteria for the experiment, i.e.: disease or functional disorders of the cardiovascular system and autonomic nervous system according to the functional assessment of the short version of autonomic symptoms profile, none of the subjects were taking any on medication [15,17]. Each subject was examined by a specialist in cardiology and physical medicine prior to the procedure.

Mean age 32.8 ± 6.9 years, mean body height and mass 1.7 ± 0.0 m and 82.0 ± 6.9 kg, mean body mass index 25.6 ± 2.9 kg/m^2, body surface area 2.0 ± 0 m^2, systolic blood pressure at rest 118.3 ± 6.2 mmHg and diastolic blood pressure at rest 75.1 ± 4.3 mmHg. More details regarding the specific characteristic are given in Table 1. The study was approved by the Human Research Committee of the Nicolas Copernicus University in Torun, The Ludwik Rydygier Collegium Medicum in Bydgoszcz, and the subjects gave their written consent to participate after being informed about the whole procedure and the study protocol. All subjects were instructed to refrain from smoking, caffeine, alcohol ingestion, and intensive physical activity on the day of investigation and ate a light breakfast only. They were also asked to avoid taking part in any exercise at least 24 h prior WBC exposure.

Cryostimulation (WBC)

Each participant was exposed to a cryotherapeutics factor (whole-body cryotherapy/cryostimulation WBC) with a temperature of approximately −120 °C for a period of 3 min. In the study a modern cryochamber divided into three compartments with different temperatures was used. First one with a temperature of −10 °C, second one with −60 °C followed by the main compartment with −120 °C (Cryotherapy chamber – "Stan-Mar", Poznan, Poland). Subjects were exposed to the cryotherapeutics stimuli in swimwear, to prevent frostbites they were equipped with headband, facemask, gloves and wooden clogs. During exposure subjects walked slowly without rapid body movements. In all subjects WBC procedure were performed between 9 am and 11 am.

Body superficial temperature assessment

The temperature of the selected areas of body surface was assessed by means of the infrared (IR) thermoimaging procedure, according to the thermal image acquisition criteria described by Ring and Ammer [18]. This method allowed us to study the intensity of body superficial cooling during WBC procedure.

A thermographic IR-camera Flir P640 was used and allowed the capture of high resolution thermographic images. The IR-camera was maintained at a distance of 1.5 m from the subject at a height of 1.5 m from the floor, in stable ambient conditions. The first measurement was performed after 60 min of the subjects adjustment to examination conditions (before-WBC), the second was taken immediately post WBC exposure with the subject and the camera positioned as described in the guidelines [18]. The post-WBC images were captured 3 h and 6 h subsequently after WBC exposure. The mean temperatures were calculated with the processing

Table 1
General characteristics of examined group $n = 30$.

Characteristic	$n = 30$ only men	
	Mean	Range
Age (years)	32.8 ± 6.9	25–49
Body height (m)	1.78 ± 0.0	1.65–1.87
Body mass (kg)	82.0 ± 6.9	62–103
Body mass index (kg/m^2)	25.6 ± 2.9	20–31
Body surface area (m^2)	2.00 ± 0.1	1.98–2.06
sBP at rest (mmHg)	118.3 ± 6.2	107.5–129.8
dBP at rest (mmHg)	75.1 ± 4.3	64.2–86.4

software utilizing a polygon figure positioned in the defined body areas (AR_0X). Certain areas were marked as below;

Anterior view:

- AR_01: chest with shoulders.
- AR_02: abdomen.
- AR_03: right upper-arm.
- AR_04: left upper-arm.

Posterior view:

- AR_05: upper back.
- AR_06: lower back.
- AR_07: right upper-arm.
- AR_08: left upper-arm.

Hemodynamic assessment

All measurements were performed with a dedicated device – Task Force Monitor (TFM, CNSystems, Medizintechnik, Graz, Austria). The main area of TFM application is as an automated and computerized beat-to-beat analysis of impedance cardiography (ICG), electrocardiogram (ECG), oscillometric and non-invasive continuous blood pressure measurement (oscBP, contBP). From these three biological signals hemodynamic and autonomic parameters are calculated. Advantages of the apparatus include the continuous (beat-to-beat), reliable and reproducible measurements of all parameters [16,19,20].

The TFM allows intervention marks to be set for defined periods for automated basic statistical analysis. Each measurement was performed continuously for 10 min after all signals stabilized, this allowed a reliable analysis of hemodynamic and baroreceptors parameters. The TFM measurements were performed four times, at following stages "before WBC", "after WBC", "WBC + 3 h" and "WBC + 6 h". The first measurement was treated as a point of reference to the rest of the three measurements. Measurements in each subject were performed at the same time of day, and took place in a neutral ambient temperature, quiet room in strictly controlled ambient conditions.

The TFM was used to monitor beat-to-beat hear rate (HR) by 3-channel ECG, beat-to-beat stroke volume (SV), stroke index (SI), cardiac output (CO), cardiac index (CI) by an improved method of impedance cardiography and beat-to-beat blood pressure by the vascular unloading technique which was corrected automatically to the oscillometric blood pressure measured on the contralateral upper-arm. Total peripheral resistance index (TPRI) was calculated according to Ohm's law. Spontaneous baroreflex sensitivity was automatically assessed by using a sequence method which is based on the computer identification in the time domain of spontaneously occurring sequences of four or more consecutive beats characterized be either a progressive rise in systolic blood pressure and lengthening in R–R interval or by a progressive decrease in systolic blood pressure and shortening in R–R interval. The slope of the regression line between systolic blood pressure and R–R interval changes is taken as an index of the sensitivity of arterial baroreflex modulation of heart rate (BRS) [16,21].

All functions of the Task Force Monitor have been assessed previously, and the instrument has already been used successfully in many advanced clinical and scientific studies [22–24].

Statistical analysis

All data are presented as means ± SD. The normality of the distribution of variables was analyzed by the Shapiro–Wilk test. The Levane's test was applied to check the homogeneity of variances in analyzed samples. The results were compared with ANOVA for multiple repeated measurements, and post hoc Benferroni's; ANOVA-Friedman's test and post hoc Dunn's test as appropriate for parametric and non-parametric analyses. All tests were assumed with statistical significance at level $\alpha = 0.05$.

Results

Body superficial temperature changes

At baseline – before-WBC there were non-significant difference between selected body areas, the differences were normal due to cutaneous and subcutaneous tissues characteristics (ranging from 29.3 ± 1.2 °C at AR_03 to 32.3 ± 0.9 °C at AR_06) (see Table 2 and Fig. 1, Fig. 2). The thermographic images showed a intense superficial temperature drop in all analyzed skin body areas after WBC exposure. The maximal temperature drop was observed at AR_04 9.2 ± 2.3 °C, whereas the minimal drop was observed at AR_05 19.5 ± 1.7 °C (see Table 2). The mean temperature values varied according to the biological characteristics but among all considered areas they were significantly lower than the before-WBC stage, $p < 0.0001$. After WBC + 3 h mean superficial temperatures returned to normal physiological values, ranging from 31.0 ± 1.0 °C at AR_07 to 33.8 ± 1.0 °C at AR_05, and in all areas they were higher than before-WBC (see Table 2). At AR_01, AR_02, AR_03 and AR_04 mean temperatures were significantly higher than before-WBC, $p < 0.05$, (see Table 2 and Fig. 1, Fig. 2). After WBC + 6 h body superficial temperatures at almost all areas were still rising comparing to WBC + 3, but it wasn't a significant increase and mean values were ranging from 31.8 ± 1.2 °C at AR_07 to 33.9 ± 1.4 °C at AR_01, similarly like after WBC + 3 h they were significantly higher than before-WBC at AR_01, AR_02, AR_03, AR_04 and AR_07, $p < 0.05$, (see Table 2 and Fig. 1, Fig. 2).

Hemodynamic changes

After whole-body cryostimulation in healthy subjects, there statistically significant changes were observed for selected cardiovascular parameters which are shown in Table 3 and Figs. 3–7. As can be seen, HR was significantly decreased after WBC exposure (from 61.7 ± 8.4 to 55.8 ± 7.8 n/min, $p < 0.0001$), but at WBC + 3 h returned to basal values and remained at the very similar level at WBC + 6 h (62.9 ± 6.2 and 62.7 ± 6.5 n/min, $p > 0.05$) (see Table 3 and Fig. 3. SV and SI significantly increased after WBC (from 102.3 ± 22.1 to 107.1 ± 19.8 ml, $p = 0.0265$; from 51.1 ± 11.5 to 53.6 ± 10.1 ml/m^2, $p = 0.0230$), and returned to basal values at WBC + 3 h and WBC + 6 h as well (104.1 ± 24.6 and 103.0 ± 20.5 ml, $p > 0.05$; 52.2 ± 13.0 and 51.7 ± 10.8 ml/m^2, $p > 0.05$) (see Table 3 and Fig. 5.). A significant decrease in CO and CI were noted also only just after WBC exposure (from 6.2 ± 1.3 to 5.9 ± 1.1 l/min, $p = 0.0215$; from 3.1 ± 0.6 to 2.9 ± 0.5 l/min, $p = 0.0308$), and in consequences of HR and SV changes

Table 2
Mean values of body skin temperature (°C) changes before-WBC, after-WBC, WBC + 3 h and WBC + 6 h, the thermographic analysis.

Body areas (°C)	Before-WBC	After-WBC	WBC + 3 h	WBC + 6 h	*p*
AR_01	31.9 ± 1.0	14.5 ± 2.0	33.5 ± 0.9	33.9 ± 1.4	0.0000
AR_02	31.5 ± 1.1	13.9 ± 2.0	33.1 ± 1.1	33.7 ± 1.5	0.0000
AR_03	29.3 ± 1.2	10.3 ± 2.1	32.8 ± 0.9	33.2 ± 1.3	0.0000
AR_04	30.9 ± 1.0	9.2 ± 2.3	32.3 ± 0.9	32.8 ± 1.3	0.0000
AR_05	32.8 ± 0.8	19.5 ± 1.7	33.8 ± 1.0	33.8 ± 1.3	0.0000
AR_06	32.3 ± 0.9	18.3 ± 1.5	33.1 ± 1.0	33.4 ± 1.3	0.0000
AR_07	29.9 ± 0.8	13.8 ± 2.2	31.0 ± 1.0	31.8 ± 1.2	0.0000
AR_08	30.9 ± 0.9	14.4 ± 2.1	31.6 ± 1.1	32.3 ± 1.4	0.0000

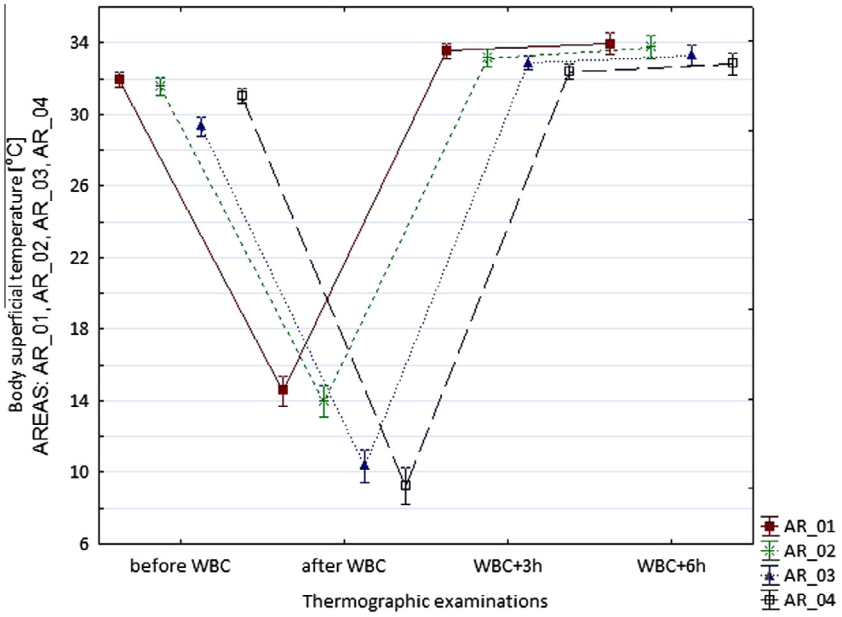

Fig. 1. Body superficial temperature (°C) before-WBC, after-WBC, WBC + 3 h and WBC + 6 h of the selected body skin areas, AR_01, AR_02, AR_03, AR_04. Values are mean ± SE.

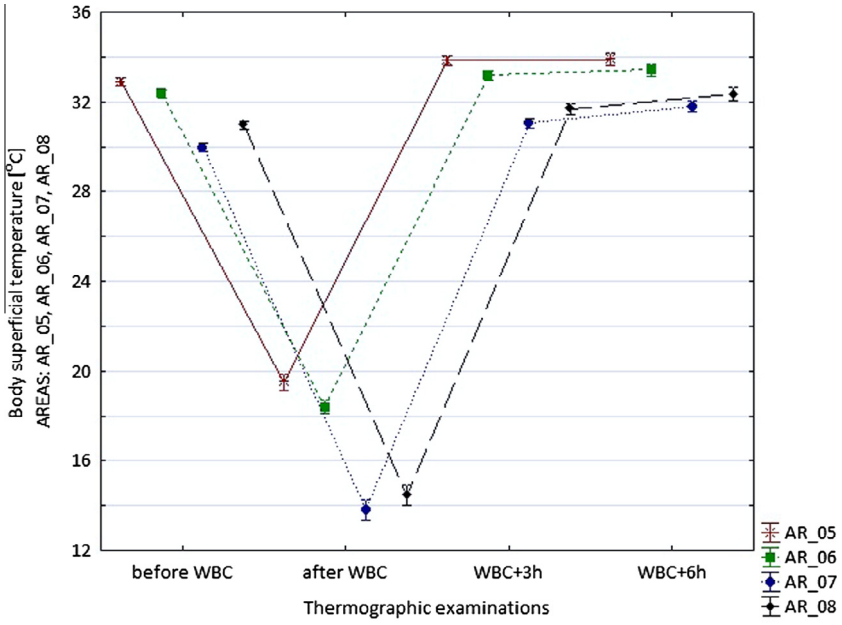

Fig. 2. Body superficial temperature (°C) before-WBC, after-WBC, WBC + 3 h and WBC + 6 h of the selected body skin areas, AR_05, AR_06, AR_07, AR_08. Values are mean ± SE.

Table 3
Mean values of hemodynamic and baroreceptors parameters before-WBC, after-WBC, WBC + 3 h and WBC + 6 h.

Parameter	Before-WBC	After-WBC	WBC + 3 h	WBC + 6 h	*p*
HR (n/min)	61.7 ± 8.4	55.8 ± 7.8	62.9 ± 6.3	62.7 ± 6.5	0.0001
SV (ml)	102.3 ± 22.1	107.1 ± 19.8	104.1 ± 24.6	103.0 ± 20.5	0.0265
SI (ml/m^2)	51.1 ± 11.5	53.6 ± 10.1	52.2 ± 13.0	51.7 ± 10.8	0.0230
CO (l/min)	6.2 ± 1.3	5.9 ± 1.1	6.5 ± 1.5	6.4 ± 1.3	0.0215
CI (l/(min ∗ m^2))	3.1 ± 0.6	2.9 ± 0.5	3.2 ± 0.7	3.2 ± 0.6	0.0308
sBP (mmHg)	118.3 ± 6.2	120.3 ± 8.5	123.1 ± 9.8	122.1 ± 8.4	0.1914
dBP (mmHg)	75.1 ± 4.3	77.3 ± 8.2	76.8 ± 7.4	77.0 ± 7.3	0.1272
mBP (mmHg)	88.3 ± 6.0	88.9 ± 8.1	90.8 ± 8.0	90.6 ± 7.9	0.6556
TPR (dyne ∗ s/cm^5)	1152.2 ± 267.9	1239.4 ± 315.9	1159.1 ± 364.9	1150.1 ± 303.1	0.0300
TPRI (dyne ∗ s ∗ m^2/cm^5)	2315.7 ± 568.6	2474.6 ± 636.8	2326.0 ± 755.9	2302.6 ± 640.6	0.0425
BRS – slope (ms/mmHg)	31.0 ± 18.7	41.1 ± 30.4	25.2 ± 13.9	23.4 ± 9.9	0.0002

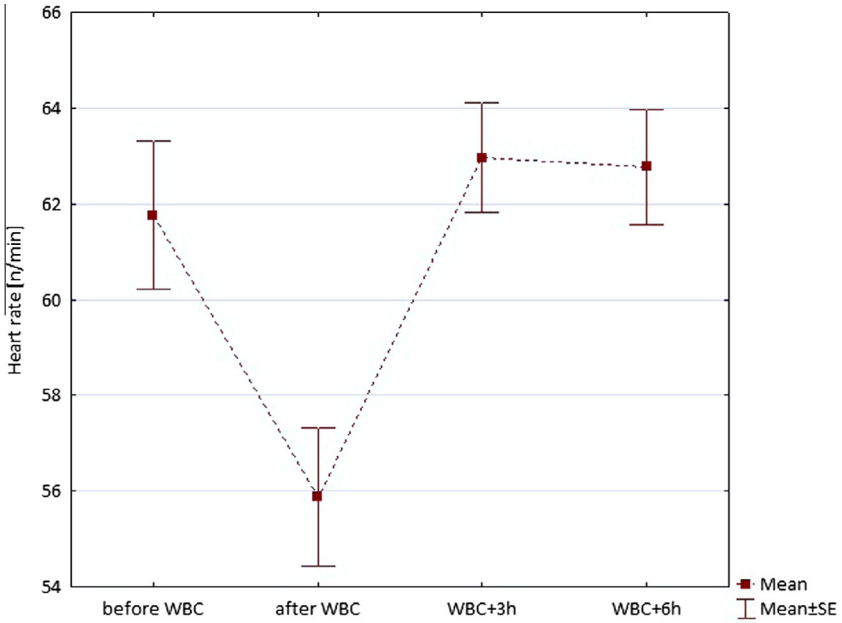

Fig. 3. Heart rate (n/min) values before-WBC, after-WBC, WBC + 3 h and WBC + 6 h.

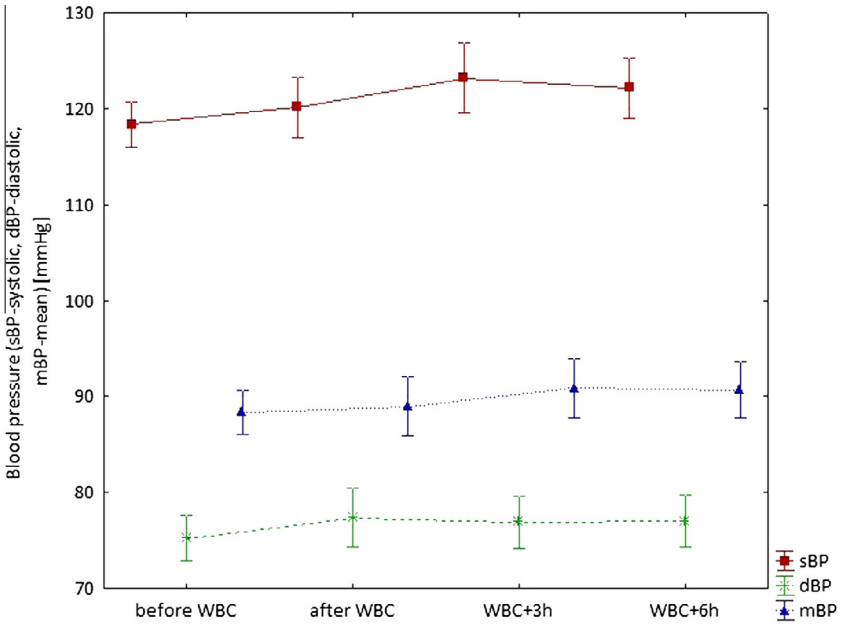

Fig. 4. Systolic, diastolic, mean blood pressure (mmHg) before-WBC, after-WBC, WBC + 3 h and WBC + 6 h.

returned to basal values at WBC + 3 h and WBC + 6 h (6.5 ± 1.5 and 6.4 ± 1.3 l/(min ∗ m^2) $p > 0.05$; 3.2 ± 0.7 and 3.2 ± 0.6 (l/min ∗ m^2), $p > 0.05$) (see Table 3). After WBC exposure was observed a slight increase but statistically significant of TPRI (from 2315.7 ± 568.6 to 2474.6 ± 636.8 dyne ∗ s ∗ m^2/cm^5, $p = 0.0425$), which wasn't noticeable at WBC + 3 h and also WBC + 6 h (2326.0 ± 755.9 and 2302.6 ± 640.6 dyne ∗ s ∗ m^2/s^5, $p > 0.05$) (see Table 3 and Fig. 6.).

Blood pressure parameters sBP, dBP and mBP slightly increased in response to the WBC procedure, although these were non-significant changes (from 118.3 ± 6.2, 75.1 ± 6.3, 88.3 ± 6.0 to 120.3 ± 8.5, 77.3 ± 8.2, 88.9 ± 8.1 mmHg, $p > 0.05$). These parameters also remained increased at WBC + 3 h and WBC + 6 h but were non-significant changes relative to basal values (123.1 ± 9.8, 76.8 ± 7.4, 90.8 ± 8.0 and 122.1 ± 8.4, 77.0 ± 7.3, 90.6 ± 7.9 mmHg, $p > 0.05$) (see Table 3 and Fig. 4.).

Baroreceptors sensitivity

After WBC exposure it was observed that the BRS significantly increased (from 31.0 ± 18.7 to 41.1 ± 30.4 ms/mmHg, $p = 0.0002$). However, BRS at WBC + 3 h was markedly depressed compared to before-WBC and after-WBC (from 31.0 ± 18.7 and 41.1 ± 30.4 to 25.2 ± 13.9 ms/mmHg, $p < 0.05$), and remained significantly decreased also at WBS + 6 h (from 31.0 ± 18.7 and 41.1 ± 30.4 to 23.4 ± 9.9 ms/mmHg, $p < 0.05$) compared to before-WBC and after-WBC but was non-significantly changed compared to

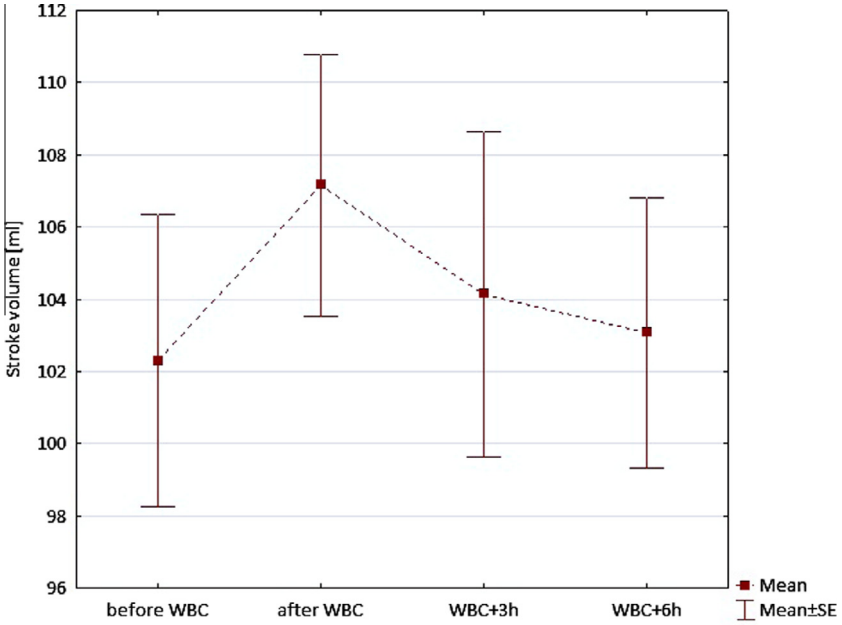

Fig. 5. Stroke volume (ml) values before-WBC, after-WBC, WBC + 3 h and WBC + 6 h.

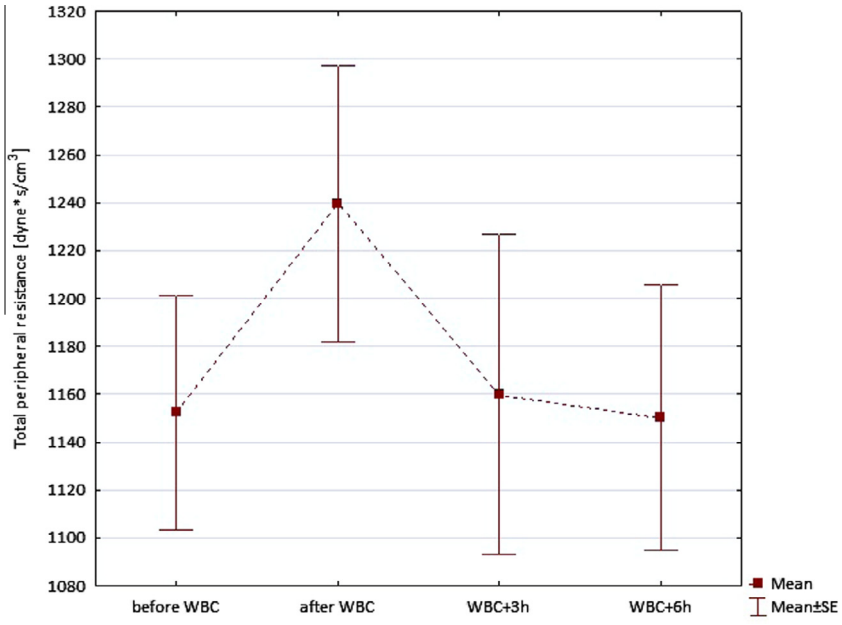

Fig. 6. Total peripheral resistance (dyne $*$ s/cm^3) values before-WBC, after-WBC, WBC + 3 h and WBC + 6 h.

WBC + 6 h (from 25.2 ± 13.9 to 23.4 ± 9.9 ms/mmHg, $p > 0.05$) (see Table 3 and Fig. 7).

Discussion

Whole body cryotherapy is a technique used predominantly in Eastern Europe that stimulates the body using intense hypothermia. Our study confirms that this technique leads to significant hemodynamic and autonomic changes that include reductions in heart rate, cardiac output, and increase in stroke volume, total peripheral resistance and baroreceptor sensitivity.

Cutaneous vessels dynamically dilate or constrict in response to changing ambient or internal body temperature, these mechanisms contribute to maintenance of a constant body temperature by controlling the heat loss or conversation. Cold exposure of the human body activate the sympathetic α-adrenergic receptors which increase a peripheral resistance by a reduction of vessels diameter. Skin sympathetic outflow is critical for thermoregulation. It includes cholinergic sudomotor, noradrenergic vasoconstrictor, and also not very well known chemically undefined vasodilator outputs [25,26].

Intense whole-body cooling during whole-body cryostimulation is a very strong sympathetic stimulation which leads to veno- and vasoconstriction to prevent heat loss and thus limits blood perfusion through the skin and increase a central blood flow through big vessels. Thermography analyses performed at each stage of the study showed dynamic changes in body skin temperature in response to a whole-body cryostimulation. After-WBC there was observed a rapid and intense skin temperature drop

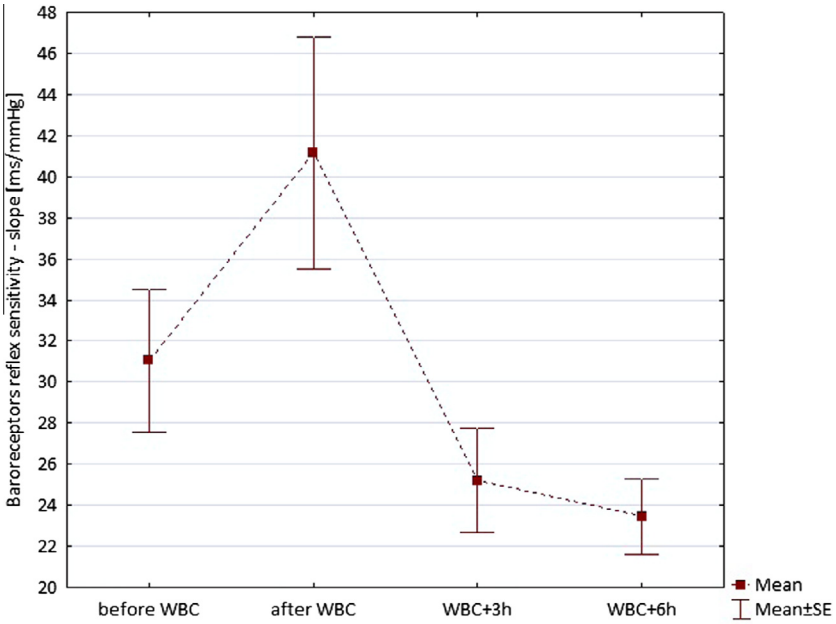

Fig. 7. Baroreceptor reflex sensitivity – slope (ms/mmHg) values before-WBC, after-WBC, WBC + 3 h and WBC + 6 h.

which is an evidence that cryotherapeutics stimulus has a very strong impact on cutaneous microcirculation. There is no other known therapeutic stimulus which cause such dynamic and intense skin cooling without any consequences upon cutaneous structures, despite the fact that the activity of skin sympathetic nerves show variability between individual subjects [27,28].

At the follow up stages, WBC + 3 h and WBC + 6 h we observed a significant increase in body skin temperature compared to before-WBC measurements. This physiological reaction is part of a systemic thermogenesis process as a response to an acute whole-body cooling. Occurrence of increased superficial temperature after 3 and even 6 h since body exposition to cryostimulation confirms that the WBC is a very strong physical stimulus which affects physiological mechanisms for next several hours even though all subjects were in an afternoon thermogenesis drop according to a body circadian cycle.

Our findings confirmed that WBC has a very strong impact on cutaneous vascular changes which lead to displace blood from cutaneous vessels into deep veins and results in systemic hemodynamic changes observed briefly after exposure [6,9,29,30].

After a whole-body cryostimulation we observed a significant reduction in heart rate (HR). It is well known that tachycardia occurs after acute local cooling, e.g., a cold pressor test [15,31], but bradycardia is present even after mild whole-body cooling [30,32]. The increase in ventricular filling as well as the flow of chilled peripheral blood into the area of atrial sinus node resulted in a significant decrease in heart rate, such a mechanism has been also confirmed by other authors [4,6,9,10].

This mechanism was also confirmed in WBC exposure, when we observed a significant reduction of heart rate just after-WBC but it wasn't present at the following stages, WBC + 3 h and WBC + 6 h. The contrary response of HR to very cold stimuli during WBC may be due to reduction of pain sensations which normally increase heart response [8,13,25,26,33]. Similar observations were also confirmed by other authors [4,6,9,10], but there are contrary reports where authors did not confirm such reactions [4,10,29]. We think that a heart rate reduction after WBC is an expect physiological reaction to a whole-body intense cooling, and described lack of heart rate changes might be due to less reliable method of HR measurements or performing the study on clinical not physiological groups which might suffer from different cardiovascular conditions [11,33].

Centralization of circulating blood after WBC causes a greater filling of large vessels and, consequently, increased ventricular filling, this is confirmed by significant change in the values of cardio-impedance parameters, i.e. an increase in left ventricular ejection SV and stroke index SI. Despite the fact that SV was increased after WBC we observed a significant decrease of cardiac output CO and cardiac index CI parameters which was caused a simultaneous decrease in the HR. Significant changes in HR and SV seem to be an essential response to acute whole-body cooling during WBC which determine following physiological reactions [9,11,12,27,28,31].

There were non-significant changes in blood pressure parameters after WBC exposure and at the following stages as well. This observations are interesting because we expected that intense sympathetic stimulation would increase these parameters. Our very detailed continuous and beat-to-beat measurement of blood pressure within a period up to 15 min after WBC exposure, showed that there were only slight increase of blood pressure parameters which were also observed at the following stages, WBC + 3 h and WBC + 6 h. Some other authors observed opposite effects of WBC, with a significant increase of blood pressure parameters [10,29,30]. However, we cannot compare these finding because these trials were performed mostly on clinical groups which contained pre- and hypertensive subjects. Such cardiovascular conditions might have a crucial role in reaction to acute whole-body cooling.

Lack of blood pressure changes was probably associated with changes of baroreceptors sensitivity (BRS). A higher degree of central blood vessel filling is reflected by direct stimulation of the arterial baroreceptor endings located in the aortic arch and carotid arteries. Physiological analysis of arterial BRS, level of sensitivity – strength of the reflex, confirmed the modulating effect of WBC on the receptors. Exposure to a cryotherapeutic agent caused a significant increase in arterial baroreceptor sensitivity, expressed as a statistically significant increase in the value of the slope of the linear regression curve of the registered baroreceptor reflex sequences. However, 3 and 6 h after WBC we observed a very significant drop of baroreceptors sensitivity, slope values were at the lower level than before-WBC and definitely lower than after-

WBC. The hypothesis of such significant changes of BRS is that might be a compensate reaction for the intense stimulation occurred after WBC exposure and increased skin blood perfusion.

It is possible that one of the factors influencing this phenomenon is greater stability of the walls of blood vessels caused by increased perfusion. Undoubtedly, the observed increase in the strength of arterial baroreceptors reflex translated into a slight increase in blood pressure parameters, which were characterized by high stability despite the influence of such intense thermal stimulus [27,28,31,34].

We also postulate that increase of baroreceptors sensitivity played an important role in HR reduction after WBC by an increase in cardiac vagal activity and it suggesting that there was a central interaction between the afferent information from arterial baroreceptors and that from skin cold receptors [28,34]. The reflex cardiac sympathetic responses to baroreceptor activation are slower than the parasympathetic responses [31,34]. Despite the fact that acute cooling during WBC is a very strong sympathetic stimulation, the intense blood centralization which rapidly affected BRS sensitivity played a main role in the vagal reduction of heart rate and non-significant blood pressure increase.

That is well known that a cold-induced peripheral vasoconstriction elevates total peripheral resistance TPR and blood pressure, and modifies capillary fluid and ion exchange between the intravascular and intrastital spaces which results in a baroreceptor inhibition of vasopressin secretion, leading to diuresis and subsequent hypovolemia [12,13,25,28]. However, this mechanism is more likely to occur after prolonged cold exposure, and WBC session does not last more than 3 min, so in this case a short term hemodynamic regulation plays an essential role. We observed a statistically significant elevation of TPR values after WBC, but the absolute TPR increase was small and inadequate to such severe cold stimulation. Little changes of TPR and TPRI might also confirm that central vagal interaction played a more important role in the presented experimental conditions [25,26,34].

In conclusion, the present findings suggest that WBC strongly stimulates the baroreceptor cardiac reflex in response to body fluid changes which sequentially modulate HR and BP control in supine resting healthy subjects. The significant decrease in baroreceptor sensitivity occurred within several hours after WBC exposure, and might be a risk factor for subjects with primarily reduced baroreceptor sensitivity.

We believe that our results are important for WBC safety determination in research and clinical studies. This study was performed on randomized and homogenic group without any clinically confirmed cardiovascular or autonomic dysfunctions, and variation of changes might be wide different in clinical groups. WBC appears to be safe for healthy subjects despite the fact of high stimulation intensity.

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