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Whole-body cryostimulation increases parasympathetic outflow and decreases core body temperature

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ABSTRACT

The cardiovascular, autonomic and thermal response to whole-body cryostimulation exposure are not completely known. Thus the aim of this study was to evaluate objectively and noninvasively autonomic and thermal reactions observed after short exposure to very low temperatures. We examined 25 healthy men with mean age 30.1 ± 3.7 years and comparable anthropomorphical characteristic. Each subject was exposed to cryotherapeutic temperatures in a cryogenic chamber for 3 min (approx. −120 °C). The cardiovascular and autonomic parameters were measured noninvasively with Task Force® Monitor. The changes in core body temperature were determined with the Vital Sense® telemetric measurement system. Results show that 3 min to cryotherapeutic temperatures causes significant changes in autonomic balance which are induced by peripheral and central blood volume changes. Cryostimulation also induced changes in core body temperature, maximum drop of core temperature was observed 50–60 min after the stimulation. Autonomic and thermal reactions to cryostimulation were observed up to 6 h after the exposure and were not harmful for examined subjects.

1. Introduction

The term "whole-body cryotherapy (cryostimulation)" (WBC) refers to an array of therapeutic applications, that utilize cryogenic temperatures. Usually, the cryogenic temperatures used during WBC range from −110 °C down to −150 °C. The treatment is based on a relatively short (usually up to 180 s) exposure of the whole body to extremely cold air, in order to achieve considerable cooling of the skin. This is associated with resultant activation of processes that lead to accumulation of heat and counterbalance its loss (Costello et al., 2012a,b,c, 2014; Banfi et al., 2010; Guillot et al., 2014).

Typically, WBC is recommended for alleviation of pain and treatment of inflammatory conditions, acute injures of soft tissues, rheumatic diseases and neurodegenerative disorders; however, it can be used in patients with depressive and anxiety disorders as well. Moreover, whole-body cryotherapy is very popular as a method of wellness treatment and athletic recovery. Initially introduced in Japan, Germany and Poland, WBC has gained increasing popularity worldwide (Costello et al., 2012b, 2014; Banfi et al., 2010; Guillot et al., 2014; Lubkowska and Szygula, 2010; Lubkowska and Suska, 2011).

Rapid cooling of virtually the whole surface of the body during WBC results in strong vasoconstriction of the skin capillaries. This is associated with enhanced venous return of cooled blood. Increased perfusion of large vessels of the trunk results in immediate activation of arterial baroreceptors and increase in

Abbreviations: ANS, autonomic nervous system BPV, blood pressure variability; BRS, baroreceptor reflex sensitivity; BSA, body surface area; dBP, diastolic blood pressure; dBPV, diastolic blood pressure variability; HF, high-band frequency spectrum; HF-dBP, HF-component of dBPV; HFnu-dBP, normalized "HF-component" of dBPV; HFnu-RRI, normalized "HF-component" of HRV; HFnu-sBP, normalized "HF-component" of sBPV; HF-RRI, HF-component of HRV; HF-sBP, HF-component of sBPV; HR, heart rate; HRV, heart rate variability; LF, low-band frequency spectrum; LF/HF, sympatho-vagal balance LF-dBPV/HF-RRI; LF/HF-dBP, LF/HF ratio of dBPV; LF/HF-sBP, LF/HF ratio of sBPV; LF-dBP, LF-component of dBPV; LFnu-dBP, normalized "LF-component" of dBPV; LFnu-RRI, normalized "LF-component" of HRV; LFnu-sBP, normalized "LF-component" of sBPV; LF-RRI, LF-component of HRV; LF-sBP, LF-component of sBPV; mBP, mean blood pressure; PSD-dBPV, power spectral density of dBPV; PSD-RRI, power spectral density of HRV; PSD-sBPV, power spectral density of sBPV; sBP, systolic blood pressure; sBPV, systolic blood pressure variability; TFM, Task Force® Monitor; WBC, whole-body cryostimulation

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parasympathetic stimulation of the heart (Zalewski et al., 2013; Hausswirth et al., 2013). Cyclic circadian changes of core body temperature constitute a well-known physiological process which is under tight control of the nervous system. As such, this mechanism can be disturbed by a number of stressors, e.g. very low temperature.

According to most authors, core body temperature does not change in response to whole-body cryotherapy (Selfe et al., 2014; Komulainen et al., 2004). However, this apparent lack of changes could also be a consequence of the exposure to the cryogenic factor being too short (no longer than 2 min), inaccurate method of measurement, and particularly characteristics of the study sample.

Thermoregulatory mechanisms are based on a tight relationship between superficial and core body temperature. WBC decreases the temperature of the skin, especially in the lower limbs (Cholewka et al., 2011). According to some authors, systemic cryostimulation does not cause significant changes in anal temperature, and the lowest local superficial temperatures are recorded on the forearms. However, data on the distribution of superficial temperatures after whole-body cryostimulation remain inconclusive (Costello et al., 2012a,b,c). Nevertheless, WBC is not associated with the risk of hypothermia (Westerlund et al., 2003).

In addition to changes in superficial body temperature, whole-body cryotherapy also affects function of the cardiovascular system. Exposure to cryogenic temperatures is reflected in a decrease in heart rate (HR) and an increase in stroke volume (SV) and stroke index (SI). In contrast, the values of systolic (sBP), diastolic (dBP) and mean blood pressure (mBP), cardiac indices (CO, CI) and total peripheral resistance (TPR, TPRI) do not alter significantly in response to WBC (Zalewski et al., 2013; Bonomi et al., 2012). The spectrum of changes in the abovementioned parameters suggests that systemic cryotherapy causes an increase in preload but does not affect afterload of the heart (Zalewski et al., 2013). However, some studies have documented a significant increase in systolic and diastolic blood pressure as a form of stress response to whole-body cryostimulation (Lubkowska and Suska, 2011). Typically, women and men did not differ significantly in terms of their response to WBC (Westerlund et al., 2004).

All reflex mechanisms of the cardiovascular system are integrated and controlled by the autonomic nervous system. Therefore, the activity of autonomic fibers that innervate the heart and blood vessels can be determined non-invasively, using heart rate and blood pressure variability.

The aim of this study was to analyze the effects of whole-body cryostimulation on core body temperature and the autonomic nervous system of healthy individuals.

2. Material and methods

2.1. Subjects

The study included the group of 25 healthy men aged between 25 and 39 years (mean: 30.1 ± 3.7 years). Anthropometric characteristics of the study participants are presented in Table 1. Any functional disorders of the cardiovascular and autonomic nervous system (ANS), and other contraindications to whole-body cryotherapy constituted exclusion criteria from the study. Each of the participants was subjected to a single 3-min procedure of whole-body cryotherapy (at a chamber temperature of between −120 °C and −110 °C). The research project was conducted in one of the Polish modern rehabilitation clinics (Rehabilitation Clinic "*Pod Tężniami*", Ciechocinek, Central Poland). Prior to the exposure, and during the post-cryotherapy period, the participants remained in a dedicated faculty for cryotherapy procedures that included an air-conditioned room with constant ambient temperature and humidity. During the whole experiment (up to 6 h after exposure) each subject stayed in a resting room which also controlled ambient conditions, and their activity was supervised by research staff. Physical activity of the subject was limited to a necessary minimum, they were allowed only to walk across the room or watch TV. Subjects did not participate in any activities which could influence natural core body temperature balance. All subjects were non-smokers and were also instructed to refrain from caffeine, alcohol ingestion, and intensive physical activity on the day of investigation and ate a light breakfast only. Food intake during the experiment was also controlled by research staff. During the whole experiment subjects were allowed to drink only water and had two light, thermoneutral meals between "after_WBC"–"WBC+3 h" and "WBC+3 h"–"WBC+6 h" stages; first water consumption was allowed two hours after telemetric capsule intake.

Table 1
Basic characteristics of the study participants (*abbreviations are listed in* Section 1).

Parameter	**Subjects *n*=25 only men**	
	Mean	**Range**
Age (years)	30.1 ± 3.7	25–39
Body height (m)	1.79 ± 0.05	1.69–1.87
Body weight (kg)	83.2 ± 11.0	62–103
BMI (kg/m^2)	25.9 ± 2.8	21–31
BSA (m^2)	2.0 ± 0.1	1.7–2.2

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.

2.2. Whole-body cryotherapy stimulation

Each participant was exposed to a cryotherapeutic factor (whole-body cryotherapy/cryostimulation, WBC) at a temperature of approximately −120 °C for a period of 3 min. A modern cryochamber (*Cryotherapy chamber – "Stan-Mar", Poznan Poland*), divided into three compartments with different temperatures (−10 °C, −60 °C and −120 °C) was used. The subjects entered the chamber in swimwear, equipped with headband, facemask, gloves and wooden clogs to prevent frostbites. During the exposure, the participants were allowed to walk slowly, avoiding any rapid body movements. All the WBC procedures took place between 9 am and 11 am.

2.3. Autonomic nervous system assessment

All measurements were performed with a dedicated device – Task Force Monitor (TFM, *CNSystems*, *Medizintechnik*, Graz, Austria). The main area of TFM application is an automated and computerized beat-to-beat analysis of impedance cardiography (ICG), electrocardiogram (ECG), oscillometric and non-invasive continuous blood pressure measurement (oscBP, contBP). These three biological signals are used for calculation of hemodynamic and autonomic parameters. The availability of continuous (beat-to-beat), reliable and reproducible measurement of all parameters represents a main advantage of the device.

Task Force Monitor allows intervention marks to be set for defined periods to allow automated basic statistical analysis. Each measurement was performed continuously for 10 min after all signals stabilized; this allowed a reliable analysis of hemodynamic and baroreceptor parameters. The Task Force Monitor measurements were performed four times, at the following stages "before WBC", "after WBC", "WBC+3 h" and "WBC+6 h". The first measurement was treated as a baseline for the remaining three

measurements. For each subject measurements were taken at the same time of day, at a neutral temperature in a quiet room with strictly controlled ambient conditions. All functions of the Task Force Monitor have been validated prior to the study, and the instrument has already been used successfully in many advanced clinical and scientific projects (Fortin et al., 1998, 2006).

2.4. Core body temperature measurements

The changes in core body temperature were determined with the Vital Sense telemetric measurement system (Equivital, Hidalgo Ltd., Cambridge, UK; formerly: Philips Respironics, Mini Mitter Co. Inc., *Bend Oregon, USA*). The Vital Sense system for telemetric measurement of core body temperature comprises two components: a portable monitor that registers, stores and exports digital records of temperature, and a telemetric capsule (*Core Body Temperature Capsule – CBTC)* (Neubert et al., 2010; Vishal et al., 2012).

After activation of the CBTC via the remote monitor, each participant swallowed the capsule with small amount of warm water (36 °C). After 1 min the capsule began registration of core body temperature communicating with the monitor using radio frequency transmission at 15-s intervals; mean values of each four consecutive measurements were recorded in the monitor's memory.

To analyze the effect of cryogenic temperatures on core body temperature, registration of the latter started at least one hour prior to the procedure of whole-body cryostimulation. The analysis included values of core body temperature documented immediately after WBC and 3 h and 6 h thereafter. Moreover, time to the maximum post-WBC decrease in core body temperature (usually 45–55 min) was recorded.

2.5. Statistics

All data are presented as means ± SD. Normal distribution of the study variables was verified with the Shapiro–Wilk test. The Levane's test was used to check the homogeneity of variances in analyzed samples. The results were compared with ANOVA and Tukey post-hoc test, or with Friedman's ANOVA test and Dunn's post-hoc test where appropriate. The results of the tests were considered significant at $\alpha = 0.05$.

3. Results

Cryostimulation was associated with significant changes in heart rate (HR) and baroreceptor sensitivity (BRS). A decrease in HR was observed immediately after WBC, with subsequent stabilization at the pre-treatment level 3 h and 6 h thereafter. In turn, BRS increased markedly immediately after cryostimulation, and then decreased below its baseline level. In contrast, we did not document any significant effects of cryostimulation on blood pressure parameters of our participants (Table 2).

A significant increase in low-(LF-RRI) and high-frequency (HF-RRI) components and power spectral density (PSD-RRI) of HRV was observed immediately after WBC, with a subsequent decrease at 3 h and another slight increase at 6 h post-exposure. Nevertheless, at the end of the study, the values of all three parameters were still significantly lower than the respective baseline levels. The post-WBC increase in parasympathetic activity was reflected by the dynamics of sympathetic–parasympathetic ratio (LF/HF), with a significant decrease in this parameter being observed immediately after cryostimulation (Table 3).

WBC resulted in a significant decrease in low-frequency components of diastolic blood pressure variability, LFnu-dBP and LF-dBP. The values of both parameters increased at 3 h post-exposure, but at 6 h LF-dBP still remained well below its baseline level. In contrast, a significant increase in both high-frequency components, HFnu-dBP and HF-dBP, was observed immediately after WBC, with a subsequent decrease at 3 h and 6 h. However, both high-frequency components of dBP variability differed in terms of their post-cryostimulation dynamics. The initial increase in HFnu-dBP was markedly more pronounced than in the case of HF-dBP; but this parameter returned to its baseline level 3 h and 6 h after the stimulation. In contrast, only a slight increase in HF-dBP was documented immediately after WBC, with a subsequent decrease at 3 h and normalization at 6 h. Furthermore, cryostimulation resulted in a persistent decrease in power spectral density of diastolic blood pressure variability, as well as in a dramatic decrease in sympathetic–parasympathetic ratio (Table 3).

A significant decrease in both low-frequency components of systolic blood pressure spectrum, LFnu-sBP and LF-sBP, was observed immediately after WBC. While the former parameter normalized at 3 h and 6 h after the stimulation, the latter remained decreased till the end of the study. The abovementioned changes in low-frequency spectral parameters were associated with a decrease in sympathetic–parasympathetic ratio (Table 3).

Cryostimulation also induced changes in core body temperature. Interestingly, this parameter remained unchanged immediately after the stimulation (after_WBC level ($P > 0.05$)). Then a significant decrease was documented 50–60 min after the stimulation, max. drop_after_WBC level (down to 36.8 °C on average, $P < 0.01$). Subsequent increase above the pretreatment level was observed at WBC+3 h and WBC+6 h levels, but only the final level of core body temperature measurement was statistically significant ($P < 0.01$) (Table 4).

The changes in the cardiovascular parameters during the research protocol prior to WBC and at various stages after the procedure are presented schematically in Fig. 1.

4. Discussion

The use of physical thermotherapy methods is widespread and well documented in literature. Nevertheless, comprehensive analysis of available data on the effects of cryostimulation on the autonomic nervous system and core body temperature points to still insufficient

Table 2
Resting values of basic cardiovascular and baroreceptor parameters in the study group; * indicates significant differences between the pre- and post-treatment parameters; all data are expressed as mean ± standard deviations, and *P* values *(abbreviations are listed in* Section 1).

Parameter	Before_WBC	After_WBC	WBC+3 h	WBC+6 h	*P*
HR (n/1)	62.1 ± 8.8	56.0 ± 7.8	63.5 ± 6.0	63.6 ± 6.8	***P* < 0.01***
sBP (mmHg)	119.7 ± 6.0	120.6 ± 5.1	124.3 ± 9.8	122.9 ± 7.9	0.0625
dBP (mmHg)	74.2 ± 5.1	76.9 ± 8.2	76.6 ± 7.5	77.8 ± 6.7	0.0932
mBP (mmHg)	87.8 ± 5.2	88.9 ± 8.2	91.1 ± 8.4	90.8 ± 7.6	0.1037
BRS (ms/mmHg)	34.4 ± 18.7	44.7 ± 31.9	27.1 ± 14.5	25.1 ± 10.1	***P* < 0.01***

Table 3
Resting values of heart rate and blood pressure variability parameters in the study group; * indicates significant differences between the pre- and post-treatment parameters; all data are expressed as mean ± standard deviations, and *P* values (*abbreviations are listed in* Section 1).

Parameter	Before_WBC	After_WBC	WBC+3 h	WBC+6 h	*P*
Heart rate variability					
LFnu-RRI (%)	52.4 ± 15.8	47.7 ± 16.5	56 ± 15.3	53.3 ± 15.6	0.1176
HFnu-RRI (%)	47.6 ± 15.8	52.3 ± 16.5	44 ± 15.3	46.7 ± 15.6	0.1176
LF-RRI (ms^2)	1679.8 ± 1337.9	2321.5 ± 2991.3	866.7 ± 807.2	1001.5 ± 894.7	**$P<0.01$***
HF-RRI (ms^2)	1742 ± 2180.5	2993.5 ± 4831.2	767.4 ± 1192.9	1132.2 ± 1658.4	**$P<0.01$***
PSD-RRI (ms^2)	4110.8 ± 3712.6	5923.4 ± 6646.2	1883.8 ± 1569.1	2454.4 ± 2427.3	**$P<0.01$***
LF/HF (n/1)	1.6 ± 2.3	0.9 ± 0.6	1.6 ± 1.4	1.4 ± 0.8	**$P<0.01$***
Diastolic blood pressure variability					
LFnu-dBP (%)	50.5 ± 15.1	38.8 ± 15.8	55.1 ± 13.9	51.8 ± 15.1	**$P<0.01$***
HFnu-dBP (%)	11.8 ± 9.7	20.7 ± 14.2	12.7 ± 10.9	12.9 ± 10.0	**$P<0.01$***
LF-dBP ($mmHg^2$)	9.5 ± 18.4	2.6 ± 2.4	4.0 ± 3.7	4.5 ± 4.8	**$P<0.01$***
HF-dBP ($mmHg^2$)	1.1 ± 0.9	1.2 ± 0.9	0.5 ± 0.3	0.9 ± 1.2	**0.0444***
PSD-dBP ($mmHg^2$)	16.4 ± 25.5	6.4 ± 4.3	6.4 ± 4.9	8.2 ± 8.0	**$P<0.01$***
LF/HF-dBP (n/1)	11.5 ± 20.5	2.8 ± 1.9	8.2 ± 7.7	7.6 ± 7.7	**$P<0.01$***
Systolic blood pressure variability					
LFnu-sBP (%)	45.9 ± 13.2	33.1 ± 15.9	45.3 ± 14.9	44.5 ± 13.4	**$P<0.01$***
HFnu-sBP (%)	15.1 ± 9.1	18.3 ± 13.7	16.6 ± 12	17.8 ± 12.7	0.6467
LF-sBP ($mmHg^2$)	12 ± 20.5	4.0 ± 6.0	5.1 ± 6.6	6.4 ± 7.5	**$P<0.01$***
HF-sBP ($mmHg^2$)	1.8 ± 1.5	1.3 ± 0.7	1.5 ± 1.3	2.3 ± 2.9	0.1332
PSD-sBP ($mmHg^2$)	22.3 ± 32.1	10.8 ± 10.1	10.2 ± 9.4	14.8 ± 16.2	0.2110
LF/HF-sBP (n/1)	7.6 ± 16.3	3.5 ± 4.5	4.1 ± 4.2	4.2 ± 4.6	**0.0325***

Table 4
Dynamics of core body temperature at various stages of the experiment.

Measurement $F=20.30$ $P<0.01$	**Core body temperature [°C]**			
	Mean	**Minimum**	**Maximum**	**SD**
Before_WBC	37.1	36.6	37.7	0.2
After_WBC	37.0	36.3	37.7	0.3
Max. drop_after_WBC	36.8	36.1	37.5	0.2
WBC+3 h	37.2	36.6	38.0	0.2
WBC+6 h	37.4	37.1	37.9	0.2

number of studies dealing with the problem in question. Growing popularity of whole-body cryostimulation, both as a clinical procedure and as a method of biological renewal, underlies why further studies in this matter are important especially those involving novel research techniques (Hausswirth et al., 2013).

In the current study, one session of whole-body cryostimulation significantly affected thermal equilibrium of healthy subjects, which was associated with compensatory changes in their cardiovascular and autonomic nervous systems (Zalewski et al., 2013). Physiological mechanisms of thermoregulation, namely sudden constriction of blood vessels of the skin, is driven by activation of sympathetic α-adrenergic receptors (especially α_2), that leads to a decrease in blood vessel diameter and marked reduction of peripheral perfusion. Previous studies have confirmed that exposure to cryogenic stimulation causes marked reduction of skin capillary perfusion, resulting from strong contracture of precapillary sphincters of low-resistance arteriovenous anastomoses, located at the point where the capillaries originate from arterioles and metarterioles (Cholewka et al., 2006, 2011). The 3-min exposure to cryogenic factors caused a shift of cooled blood into deeper located vessels, which was associated with a significant decrease in the core body temperature of our participants. The maximum decrease in core body temperature was observed approximately 50–60 min post-exposure. The presence of such a several-minute-long, latency period has its physiological explanation. This mechanism, referred to as "*afterdrop*", can be observed as a consequence of rapid body cooling, e.g. during whole-body cryostimulation (Brazaitis et al., 2014; Westerlund et al., 2003). Physiological consequences of body cooling include gradual normalization of superficial body temperature at its pre-exposure level, and simultaneous decrease in core body temperature. According to some authors, the hypothermia-induced decrease in skin perfusion persists throughout the entire afterdrop process (Costello et al., 2012a,b,c; Janský et al., 2005). Although the decrease in the body temperature of our participants turned out to be statistically significant, it was still acceptable from a viewpoint of physiological thermoregulation, and was not associated with the risk of hypothermia. Interestingly, the decrease in core body temperature was observed in spite of performing all the experiments in the morning i. e. at the time when one could expect a physiological increase in internal temperature. Furthermore, it is noteworthy that during the normalization phase, core body temperature increased above its pre-exposure level, while one would rather expect it to decrease in association with circadian rhythms. This phenomenon was probably associated with a negative feedback loop of the hypothalamic thermoregulatory center. After receiving an error signal, i. e. dominant signaling from a given type of thermal receptors, the hypothalamic thermoregulatory center activates mechanisms of heat loss or accumulation (Stocks et al., 2004; Westerlund et al., 2004; Potkanowicz et al., 2003). Therefore, the increase in core body temperature of our participants, observed 3 h and 6 h post-exposure, most likely reflected a significant disturbance of thermal equilibrium.

Noticeably, other authors have not documented changes in core body temperature after exposure to cryogenic factor. A number of reasons might explain these differences, e.g. differences in methodology or accuracy of core body temperature measurements, number of examined individuals and/or their anthropometric characteristics. Moreover it is possible that a latency period preceding the activation of hypothalamic set point by extrinsic thermal stimulation; such as documented in our study, in which the significant decrease in core body temperature was observed nearly one hour post-exposure, rather than immediately thereafter (Selfe et al., 2014; Komulainen et al., 2004).

A significant decrease in heart rate was most evident in the cardiovascular response to cryogenic stimulation observed in our participants. This finding is consistent with the results published by other authors (Bonomi et al., 2012; Lubkowska and Szygula, 2010; Westerlund et al., 2006). Two mechanisms could be

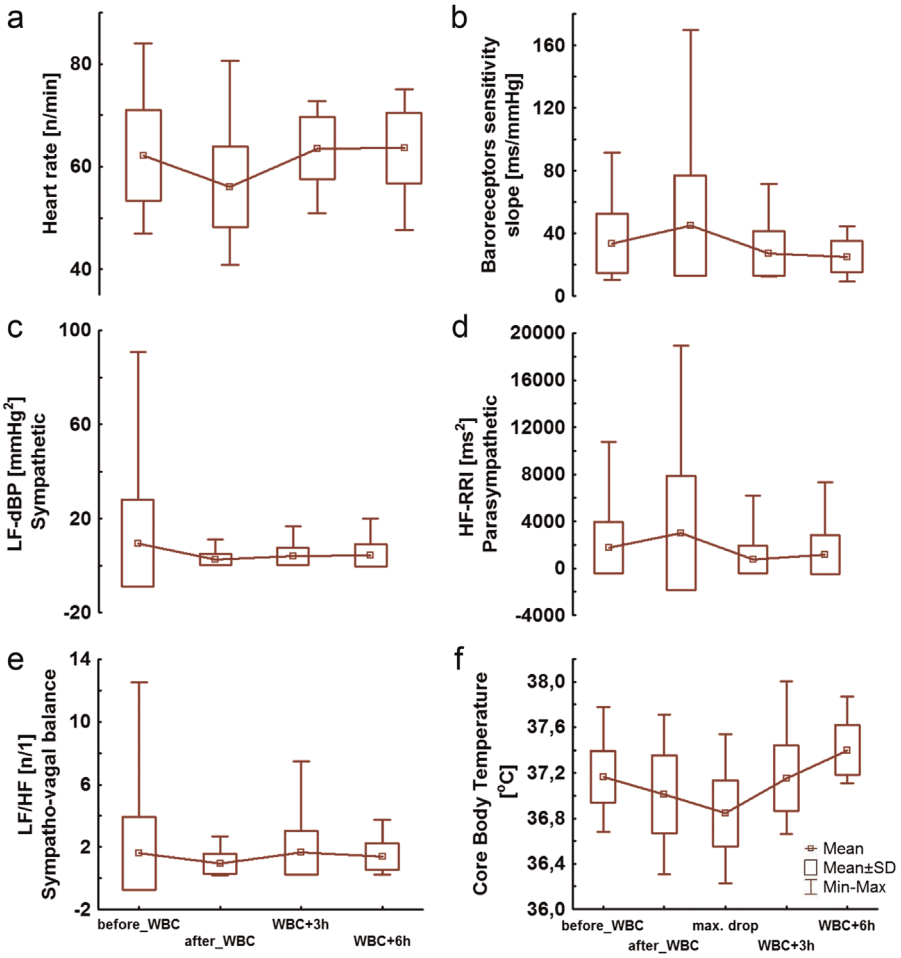

Fig. 1. Changes in parameters during research protocol: (a) heart rate, (b) baroreceptors reflex sensitivity, (c) low-frequency of diastolic blood pressure spectral variability, (d) high-frequency of heart rate spectral variability, (e) sympatho-vagal balance and (f) core body temperature, prior to WBC (before_WBC), immediately after (after_WBC), 3 h (WBC+3 h), and 6 h after the WBC procedure (WBC+6 h). (f) Illustrates maximum post-WBC decrease in core body temperature 50–60 min after WBC exposure.

responsible for the post-cryostimulation decrease in heart rate. The first is a decrease in the sinoatrial node (SAN) rate resulting from the inflow of cooled blood, i. e. negative chronotropic effect. The second is associated with enhanced parasympathetic stimulation, leading to a decrease in heart rate due to activation of baroreceptors and vagus nerves. The activation of the latter mechanism in our participants was confirmed by the significant increase in arterial baroreceptor sensitivity, resulting from enhanced venous return and increased perfusion of large vessels (Yamazaki and Sone, 2001). Moreover, the activation of baroreceptors was responsible for the lack of change in arterial blood pressure of our subjects; one could rather expect an increase in blood pressure, as cryostimulation stimulates adrenergic activity, at least during the early stages of the exposure. This is a possible explanation for the contradictory findings, i. e. an increase in both heart rate and blood pressure, reported by other authors; these discrepancies could result from different methodology of measurements, namely determining the cardiovascular parameters immediately after the exposure, when the response of participants was predominated by stress, or from longer latency period of the SAN rate modulation by cooled blood (Westerlund et al., 2004; Sawasaki et al., 2001).

The results of our spectral analyzes of heart rate variability (HRV) and blood pressure variability (BPV) confirmed that whole-body cryostimulation is a strong modulator of autonomic activity.

The use of spectral analysis of HRV and BPV for interpretation of functional changes in ANS has its several-year-long history, and a number of authors have confirmed high reliability and sensitivity of this method in determination of sympathetic–parasympathetic balance. It should be noted, however, that the interpretation criteria of spectral analyzes of heart rate and blood pressure variability are still a matter of discussion (Task Force, 1996; Malliani et al., 1994). The results of the spectral analysis of HRV and BPV confirmed our findings reported above. It is noteworthy that the HF-RRI component of HRV spectrum, a measure of parasympathetic activity, and LF-dBP component of dBPV spectrum, a marker of sympathetic activity, are the most useful indices of changes in sympathetic–parasympathetic balance (Freeman, 2006; Mäkinen et al., 2008).

After cryostimulation, HRV spectrum is characterized by an increase in high-frequency component (HF-RRI), corresponding to enhanced parasympathetic response. HF-RRI reflects spectral changes associated with activation of baroreceptors, and its values are specific for parasympathetic influence on SAN (Mäkinen et al., 2008; Cui et al., 2007). As mentioned previously, enhanced venous return causes stimulation of carotid and aortic baroreceptors, which is reflected by parasympathetic inhibition of SAN; the increase in HF-RRI documented in our study seems consistent with this sequence of changes. In turn, the changes in low-frequency component (LF-RRI) reflect stimulation of SAN by efferent sympathetic neurons and in part by the parasympathetic fibers as well, albeit involvement of the latter was not unambiguously confirmed to date. Nevertheless, the post-exposure increase in LF-RRI documented in our study was not as pronounced as in

the case of HF-RRI. The post-WBC increase in power spectral density (PSD-RRI) resulted predominantly from the elevation of HF-RRI; this hypothesis is supported by a concomitant decrease in sympathetic–parasympathetic ratio (LF/HF) and changes in normalized components of HRV spectrum (LFnu-RRI and HFnu-RRI). Importantly, a significant decrease in power spectral density (PSD-RRI), both for LF-RRI and HF-RRI, was observed at further stages of the experiment, namely 3 and 6 h post-WBC, along with simultaneous normalization of LF/HF ratio at its baseline level. These changes in spectral parameters of HRV suggest that parasympathetic predominance is observed solely immediately after WBC. In contrast, no enhancement of parasympathetic activity was documented at subsequent stages of the experiment. The significant decrease in total power spectral density (PSD-RRI) at further stages of our study can be interpreted as a consequence of decrease in the activity of both sympathetic and parasympathetic component. The results of spectral analysis of HRV were in line with the changes of HR, sBP, dBP, mBP and BRS observed during consecutive stages of our experiment, and the relationships documented herein were previously reported by other authors (Mäkinen et al., 2008; Westerlund et al., 2004, 2006).

In line with the changes documented on spectral analysis of HRV, we observed a significant decrease in power spectral density of diastolic blood pressure variability dBPV (PSD-dBP) and low-frequency component of dBPV spectrum (LF-dBP), and a slight increase in HF-dBP. These changes of spectral dBPV parameters most likely reflected the centralization of circulation associated with enhanced venous return. Increased perfusion of large blood vessels of the chest was reflected by stronger influence of hydrostatic pressure and lower fluctuation of blood pressure, thus directly attributing to the decreased level of PSD-dBP. The spectrum of changes in sympathetic–parasympathetic ratio of dBPV (LF/HF-dBP) was similar to that documented in the case of HRV, and also corresponded to parasympathetic predominance (Hausswirth et al., 2013; Mäkinen et al., 2008).

Importantly, analyzing the modulatory effects of low-temperature stimulation on ANS activity, one should consider both physical characteristics of a given stimulus (e.g. cold air blow vs. immersion of body parts in cold liquids), and duration of the exposure. Another important criterion is the intensity of stimulation, namely temperature. Finally, many authors emphasized that the response to a whole-body stimulation may differ markedly from that observed after a local stimulation.

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