



## Impact of 10 Sessions of Whole Body Cryostimulation on Cutaneous Microcirculation Measured by Laser Doppler Flowmetry

by

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*The aim of the present study was to evaluate the basic and evoked blood flow in the skin microcirculation of the hand, one day and ten days after a series of 10 whole body cryostimulation sessions, in healthy individuals.*

*The study group included 32 volunteers – 16 women and 16 men. The volunteers underwent 10 sessions of cryotherapy in a cryogenic chamber. The variables were recorded before the series of 10 whole body cryostimulation sessions (first measurement), one day after the last session (second measurement) and ten days later (third measurement). Rest flow, post-occlusive hyperaemic reaction, reaction to temperature and arterio-venous reflex index were evaluated by laser Doppler flowmetry.*

*The values recorded for rest flow, a post-occlusive hyperaemic reaction, a reaction to temperature and arterio-venous reflex index were significantly higher both in the second and third measurement compared to the initial one. Differences were recorded both in men and women. The values of frequency in the range of 0,01 Hz to 2 Hz (heart frequency dependent) were significantly lower after whole-body cryostimulation in both men and women. In the range of myogenic frequency significantly higher values were recorded in the second and third measurement compared to the first one.*

*Recorded data suggest improved response of the cutaneous microcirculation to applied stimuli in both women and men. Positive effects of cryostimulation persist in the tested group for 10 consecutive days.*

**Key words:** cryotherapy, skin blood flow, rest flow, post-occlusive hyperaemic reaction, arterio-venous reflex index

### Introduction

Whole body cryotherapy (WBCT) is more and more frequently used to complete pharmacotherapy and kinesiotherapy that are applied in rheumatologic and neurological diseases as well as in therapy of injuries of the locomotor system or in overload syndromes. It is also a modern, effective and safe procedure for athletes' recovery (Hubbard et al., 2004).

The procedure of whole body cryostimulation is based on exposure of the organism to extremely low temperature (-110°C to -160°C) for a very short period (1 – 3 minutes) without provoking hypothermia or congelation

(Westerlund et al., 2003). Cryogenic temperatures trigger physiological thermoregulation mechanisms, which results in analgesic (Long et al., 2005; Brandner et al., 1996; Ingersoll et al., 1991), anti-inflammatory (Banfi et al., 2010; Knight, 1995), anti-oedematic (Meeusun et al., 1998) and anti-oxidative effects (Akhalaya et al., 2006; Dugue et al., 2005) and stimulate the immune system (Lubkowska et al., 2010b). The effect of low temperature is especially pronounced in skin microcirculation of upper and lower limbs. After exposure to extreme cold, in the first phase constriction of skin vessels and

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opening of arterio-venous shunts occurs, due to increased sympathetic stimulation, presynaptic norepinephrine release and increased affinity of the postsynaptic  $\alpha_2$  receptors (Flouris and Cheung, 2009; Charkoudian, 2003; Chotani et al., 2000; Stephens et al., 2001; Koganezawa et al., 2006). Most of the blood flows to the body cavities in order to minimize heat losses. The skin blood flow is significantly decreased and may be completely reduced (Charkoudian, 2003). Several seconds after cessation of the stimulus, reflexive hyperaemia of the tissues occurs due to decreased sympathetic stimulation and local mechanisms – mainly accumulation of metabolites in previously hypoperfused areas as it was confirmed by studies using thermovision (Bauer et al., 1997).

Active reperfusion of the skin microcirculation persists for several hours after single cryotherapy procedure. Although the effect of a series of whole body cryotherapy sessions remains unknown. Therefore the aim of the present study was to evaluate the basic and evoked blood flow in the skin microcirculation of the hand one day and ten days after a series of 10 WBCT sessions in healthy individuals.

## Material and methods

The study group included 32 healthy, nonsmoking volunteers, students of physical education at the Opole University of Technology - 16 women (F) and 16 men (M), who had never experienced cryotherapy before. Prior to the test they had a medical examination to rule out

contraindications for cryonic sessions.

Basic anthropometric measurements were performed prior to the test. Body weight and composition were evaluated with the use of electric impedance (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 (Poland). The procedures took place once a day in the morning from Monday till Friday, for 3 minutes in the temperature of  $-130^{\circ}\text{C}$ . Each session was preceded by adaptation in the temperature of  $-60^{\circ}\text{C}$  for 30 seconds. Participants' dressing was consistent with regulation for cryonic procedures (swimsuits, covered feet, hands, ears and airways).

### *The test of selected variables of skin microcirculation.*

The microcirculation was measured using Doppler laser flowmeter Perifluks 4001 (Perimed, Sweden). Laser-Doppler measurement of cutaneous microvascular perfusion in humans has many advantages: the measurement is continuous, non-invasive and specific to the cutaneous microcirculation. The technique applied in the instrument uses the laser light of wavelength 780 nm. With this technique the laser light is used to transilluminate approximately one cubic millimeter of skin tissue and Doppler principle is adopted to measure the velocity of red blood cells in skin microvasculature.

**Table 1**  
*Somatic characteristics of the female and male study participants*

<b>Variables</b>	<b>Male n=16 (x±SD)</b>	<b>Female n=16 (x±SD)</b>
AGE [years]	21.69±1.89	20.13±0.95
BODY HEIGHT [cm]	190.56±5.20	162.46±6.41
BODY MASS [kg]	84.87±8.74	53.65±7.94
BODY MASS INDEX [kg x cm <sup>-2</sup> ]	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

The LDF signal is a stochastic representation of the number of moving cells in the tissue volume multiplied by their velocities. The flow was measured in conventional Perfusion Units score (PU), in proportion to the energy of the Doppler signal. 1 PU corresponds to the voltage of 10 mV at the outlet (Sundberg, 1984; Cankar and Strucl, 2008). The test was performed in horizontal position (on the back), in constant surrounding temperature of  $21^{\circ}\text{C}\pm 1,2^{\circ}\text{C}$ , air humidity 40-60%, after ca. 20 minutes of adaptation (Beradesca et al., 2002; Fullerton et al., 2002). The optode was placed on the skin of the back of the hand between the first and second metacarpal bones using special both sides adhesive ring. The tested skin area was healthy and shaved (Johnson and Kellogg, 2010). The participants were asked not to take part in physical activities and to avoid products that influence the circulation (coffee, tea and Coca-Cola) for at least 6 h prior to the study. Directly before the start of the test the volunteers were instructed to keep the lying position, not move and keep a steady breathing pattern (Looga, 2005).

The study protocol:

1. The procedure was started after 20 minutes of stabilization of the circulatory system in the horizontal position.
2. Blood pressure measurement (mmHg) was performed on the brachial artery.
3. Registration of the rest flow (RF) in horizontal position, on a dominating upper limb, registration time 4 min.
4. Registration of the flow after occluding the arm with the cuff of the manometer filled with air up to pressure exceeding the formerly measured systolic pressure by 50 mmHg, biological zero (BZ), registration time 4 min.
5. Registration of the reactive hyperemia (RH) after loosening the cuff, registration time 4 min.
6. Stabilization of the blood flow back to rest values.
7. Raising the optode's temperature up to  $44^{\circ}\text{C}$ , using the built-in heating module, 1 min.
8. Registration of thermal hyperemia (TH), registration time 4 min.
9. Stabilization of the blood flow back to rest values.
10. Changing the position from horizontal to vertical.
11. Registration of the RF in a standing position, after 2 minutes adaptation (ST).

Rest flow (RF), hyperaemic (RH), hyperthermic (TH) reactivity of skin microcirculation and arterio-venous reflex index (VAR) were evaluated. The arterio-venous reflex index calculated according to the formula  $\text{RF} - \text{ST}/\text{RF} \times 100\%$ . The variables were recorded before the series of 10 WBCT sessions (first measurement- I), one day after the last session (second measurement-II) and ten days later (third measurement-III).

Frequency of signals (FS) received by means of the laser Doppler fluximetry between 0.01 up to 2 Hz during basic flow was also analyzed. In this range five groups were singled out: I – frequency band between 0.01-0.02 Hz; II – frequency band between 0.021-0.05 Hz; III – frequency band between 0.051-0.145 Hz; IV – frequency band between 0.15-0.5 Hz; V – frequency band between 0.01-0.02 Hz. In each band range there is a different factor which determines blood flow oscillation. I – shows vascular oscillations depending on the endothelium metabolic activity (EF); II – shows the effect of the sympathetic system on skin flow (SF); III – illustrates oscillations resulting from the arteriola basic systolic tonus which occurs due to discharges of particular myocytes forming a circular layer of the vessel muscle coat, this response is often referred to as myogenic and it is independent of the sympathetic system (MF); IV – breath frequency (BF); V – heart frequency (HF). Time of 0.03 s was selected, and every blood flow signal was taken at the frequency of 32 Hz (Kvernmo et al., 1999). Apart from frequency, the signal power (SP) was also analyzed.

The distribution of dependent variables was tested with the use of the Shapiro-Wilk test. The differences between the tested variables in specific periods (dependent variables), as well as between men and women (independent variable), were evaluated with the use of analysis of variance (ANOVA) with repeated measurements. In case of significant effects the differences among the medians were evaluated with the post hoc multiple comparisons test (Tukey test). The values of the variables are shown as mean ( $\bar{x}$ )  $\pm$  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 consents. The experiment was accepted by Bioethical Committee of the Regional Medical Council in Opole (Resolution No 163/2009).

## Results

After a series of WBCT the resting flow (RF) increased significantly both in women ( $p=0,0001$ ) and in men ( $p=0,01$ ). After subsequent 10 days the values of RF decreased insignificantly compared to values recorded right after the procedures. No interactions between men and women were noticed.

Biological zero did not change significantly at any stage.

A statistically significant increase of mean values of post-occlusive hyperaemic reaction was observed during the second measurement in both groups: women ( $p=0,0001$ ) and men ( $p=0,0001$ ).

Although the increase in women was significantly higher than in men ( $p=0,0005$ ). The mean values of third measurement did not alter significantly compared to the second measurement. The values of HRmax were also significantly increased at the second measurement in both groups (women-  $p=0,0001$ , men - $p=0,0001$ ), whereas after 10 days the values fell in women and rose in men. The interaction between men and women was statistically significant  $p=0,01$ .

After a series of WBCT, there was a significant increase of mean and maximal values of microcirculation's reaction to temperature (mean values: women-  $p=0,0001$ , men -  $p=0,0001$ ). The third measurement showed an insignificant increase of the studied variable in men and similarly insignificant decrease in women, compared to values from the second measurement. The interaction was not significant ( $p=0,08$ ). In turn, the maximal values increased in women and decreased in men. Interaction was significant ( $p=0,00005$ ).

**Table 2**  
Mean values ( $\bar{x}$ ) and standard deviation (SD) of the analyzed variables in succeeding measurements for both sexes with statistical verification

Variables [VU]	Sex	I measurement ( $\bar{x} \pm SD$ )	II measurement ( $\bar{x} \pm SD$ )	III measurement ( $\bar{x} \pm SD$ )
RF	F	10.32±2.56	10.89±2.31*	10.11±3.17
	M	13.67±3.19	14.25±4.93*	13.83±4.68
BZ	F	2.77±0.11	2.69±0.24	2.81±0.41
	M	2.66±0.21	2.48±0.06	2.41±0.52
RH	F	75.13±17.99	108.85±33.88*	110.84±30.02**
	M	101.44±27.42	121.93±38.41*	123.94±40.42**
RHmax	F	88.56±23.26	117.32±38.93*	119.43±41.44**
	M	127.56±34.81	149.32±46.82*	148.77±39.88**
TH	F	143.88±43.9	186.24±37.77*	180.38±40.81**
	M	171.21±49.11	206.02±45.02*	209.01±30.86**
THmax	F	169.42±49.11	194.82±44.91*	199.52±36.77**
	M	196.34±58.11	229.02±51.72*	222.09±34.45**
VAR [%]	F	38.44±4.97	49.38±5.99*	50.1±4.75**
	M	42.33±5.08	55.42±6.12*	57.19±6.39**

\* statistically significant differences between I and II measurement

\*\* statistically significant differences between I and III measurement

RF – mean values of rest flow; BZ – mean values of biological zero

RH – mean values of reactive hyperemia

RHmax – maximal values of reactive hyperemia

TH – mean values of thermal hyperemia

THmax – maximal values of thermal hyperemia

VAR –arterio – venous reflex index

**Table 3**  
*Mean values (x) and standard deviation (SD) of the variables analyzed in basal flow  
 in particular frequencies in succeeding measurements  
 for both sexes with statistical verification*

		I measurement		I measurement		I measurement	
		Frequency of signals [cycles/min]	Signal power	Frequency of signals [cycles/min]	Signal power	Frequency of signals [cycles/min]	Signal power
HF	F	71.87±3,19	0.64±0,22	65.22±3.44*	0.69±0.11	66.19±3.01**	0.68±2.89
	M	68.83±5.12	0.7±0.31	62.6±4.66*	0.73±0.19	62.9±3.87**	0.71±1.37
BF	F	12.81±0.87	0.51±0.19	13.03±2.12	0.51±0.17	13.01±1,01	0.49±0.1
	M	12.02±0.59	0.49±0.2	12.73±0.72	0.47±0.11	12.71±0.91	0.5±0.09
MF	F	5.95±0.49	1.64±1.02	6.76±0.77*	1.66±0.9	6.81±0.64**	1.54±0.91
	M	6.11±0.71	1.73±0.99	6.81±0.56*	1.69±0.85	6.8±0.59**	1.69±0.75
SF	F	1.9±0.46	0.52±0.31	2.04±0.62*	0.5±0.39	2.0±0.57	0.52±0.36
	M	2.0±0.41	0.46±0.26	2.03±0.44	0.49±0,33	2.05±0.49	0.49±0.31
EF	F	0.9±0.0	3.82±1.03	0.9±0.0	4.01±1.57	0.9±0.0	3.92±1.35
	M	0.9±0.0	3.87±0.97	0.9±0.0	3.79±1.06	0.9±0.0	3.99±1.76

\* statistically significant differences between I and II measurement

\*\* statistically significant differences between I and III measurement

HF – mean values of heart frequency; BF – mean values of breath frequency

MF – mean values of myogenic frequency

SF – mean values of sympathetic frequency

EF – mean values of endothelium frequency

The arterio-venous reflex index (microcirculation's reaction to change of position) was significantly higher after WBCT in both groups (women-  $p=0,0001$ , men -  $p=0,0001$ ). Ten days later men showed further significant increase compared to the second measurement ( $p=0,01$ ). In women the increase was insignificant. The interaction between men and women was significant ( $p=0,00018$ ). The recorded results (values recorded for post-occlusive hyperaemic reaction, reaction to temperature and the arterio-venous reflex index) are shown in Table 2.

The myogenic frequency (MF) significantly rose in the second measurement compared to the first one in both men and women ( $p=0,0001$ ) and on the third measurement did not change significantly with values close to those obtained during the second measurement.

Heart frequency (HF) decreased remarkably after cryostimulation (F, M  $p=0,0001$ ) and remained stable after ten days in both groups.

Neurogenic rhythm (SF) increased significantly after cryostimulation in women

( $p=0,016$ ).

Other variables did not alter significantly. The recorded results (the values of frequency in the range of 0,01 Hz to 2 Hz) are shown in Table 3.

## Discussion

Despite the fact that WBCT has become an accepted physiotherapy method, it remains unclear how the extremely low temperatures work, what is the optimal number of sessions and how long the positive therapeutic effects for the cutaneous microcirculation last. In healthy individuals the analgesic and relaxing effects persist for several months after a series of cryotherapy sessions (Zagrobelyny and Zimmer, 1999; Wojtecka-Lukasik et al., 2010). In this study 10 cryogenic sessions were applied during 10 consecutive days as this is the most frequently applied procedure, recommended by the producers of cryogenic chambers, which does not mean it is optimal. Lubkowska et al. (2010a) proved that best therapeutic effect was observed after 20 sessions. The flows in the cutaneous

microcirculation were measured with the use of laser Doppler flowmeter. It is a safe, non-invasive and reproducible method and it records data from thermoregulation area of the vessel bed excluding the tissues underneath (Rousti et al., 2010; Sokolnicki et al., 2009). No reference values have been determined for this method, therefore the status of the skin vessel bed is defined with the use of provoked reactions to heat, occlusive or orthostatic stimulus. Many authors studied changes in skin microcirculation after local cooling but there are no data regarding the impact of extreme low temperature (WBCT) in this area.

The results obtained by the authors showed that a series of 10 WBCT sessions did not lead to significant changes in basal blood flow measured one and ten days after the sessions. The values were in the range of 10 – 20 PU, presumed by Oimomi et al. (1985) as normal. In women the values of baseline RF were lower than in men, which was previously reported by other authors (Pollock et al., 1993; Maurel et al., 1991).

Authors' own research showed significantly increased values of post-occlusive hyperaemic reaction after a series of WBCT in the second and third measurement that was similar in both female and male group. The mechanism of a hyperaemic reaction to occlusion is similar to the response to extremely low temperatures. Initially, hypoperfusion of the tissues occurs, leading to a decrease in  $pO_2$  and accumulation of the metabolites that results in active hyperaemia after cessation of the stimulus (Struel et al., 1994). WBCT provokes active reperfusion of the skin microcirculation of the whole body of much more profound intensity than in case of local occlusive stimulation. It can be presumed that a better reaction of the skin microcirculation to occlusion in the second and third measurement resulted from increased reactivity of the smooth muscles of the vessels that adapted their function to cryogenic temperatures applied. Increased frequency in the range of 0,051 – 0,145 Hz suggests that extremely low temperature impacts the myogenic control of the blood flow, although the mechanism of the adaptation requires further studies.

Authors' own research used heat stimulus of 44°C as this temperature warrants maximal response of the skin vessel bed (Charkoudian, 2003). In the first phase a rapid vasodilation

occurs due to heat dependent decreased sympathetic stimulation, later hyperaemia is sustained by increased NO secretion (Brothers et al., 2010; Rousti et al., 2010; Minson et al., 2001). Significantly higher values of hyperaemic reaction to heat were recorded after a series of WBCT compared to initial values. It can be explained by a more efficient response of the cutaneous microcirculation to decreased sympathetic stimulation. Better reactivity of the microcirculation may also explain a 50% decrease in blood flow in the arterio-venous reflex index. Change of the position from horizontal to vertical is a strong stimulus provoking vasoconstriction in order to avoid a rapid and excessive increase of hydrostatic pressure.

There is a remarkable decrease in frequency in the range of 0,01 – 0,02 Hz. Most probably it results from a move of the autonomic balance towards parasympathetic component. Such a reaction to extremely low temperature was reported by Lee et al. (2011).

The cryostimulation-related increase in neurogenic rhythm noted only in women can be explained by higher levels of estrogens. The estrogens induce increased susceptibility of the  $\alpha_2$  adrenergic receptors by increasing the oscillation neurogenic (Colucci et al., 1982).

The variations between men and women are most likely related to the hormonal differences. Over the last decade, the impact of sex hormones on skin microcirculation in women and men have been analyzed. Most studies were concentrated on estrogens, which decrease the number of angiotensin type 1 receptors (AT1) and the concentration of angiotensin convertase inhibitors (ACE) that results in vasodilation. Estrogens modulate the function of the baroreceptors and ion channels ( $K^+$ ,  $Ca^{2+}$ ) as well as increase endothelium-dependent vaso-dilating mechanisms by regulating the expression of genes of nitric oxide synthase (eNOS) or endothelin-1. They also stimulate the synthesis of prostaglandins (PG) and increase the susceptibility of the vessels to acetylcholine (Ach). Progesterone and testosterone also influence sex-related regulation of the vessel bed but their role remains controversial. Some authors report their vasodilating function whereas other suggest their vasoconstrictive effects (Mercurio et al., 1999; Sudhir et al., 1996; Huang and Kaley, 2004;

Gonzales et al., 2008).

There is no doubt that the activity of sex hormones has an impact on flows in the skin microcirculation and on reactivity of the skin vessel bed and that the differences in their concentration are probably responsible for the interaction between the groups of men and women. Although the impact of hormones on circulation in women and men still remains unclear and warrants further studies.

The values recorded in the second and third measurement were very similar and significantly higher compared to the first measurement, which suggests positive impact of

10 sessions of WBC on skin microcirculation and persistence of the effect for the following 10 days. Lack of similar studies makes a comparison difficult and shows the need for further studies.

## Conclusions

A series of 10 procedures of whole body cryostimulation resulting in a significant increase of post-occlusive hyperemic reaction, hyperemic reaction to temperature and orthostatic reaction in both women and men suggests positive effects of extremely low temperatures on reactivity of the skin microcirculation in healthy persons, persisting during whole cryostimulation period.

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