



## Changes in lipid profile in response to three different protocols of whole-body cryostimulation treatments <sup>☆</sup>

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### ABSTRACT

Systemic cryostimulation is useful treatment, both in sport and medicine, during which human body is exposed to very low, cryogenic temperature (below  $-100\text{ }^{\circ}\text{C}$ ). Although there exists some evidence of its beneficial effect in biological regeneration, so far it has not been unequivocally determined if the positive effect of repeated stimulations depends on their number in a series. The aim of this research was to estimate the influence of 5, 10 and 20 sessions of 3 min-long exposures to cryogenic temperature ( $-130\text{ }^{\circ}\text{C}$ ) on the lipid profile in physically active men. Sixty-nine healthy volunteers participated in the study. The blood samples were taken in the morning, after overnight fasting, before the first cryostimulation session, and the following morning after the last one (5th, 10th, 20th).

In serum specimens the concentration of total cholesterol (TCh), HDL cholesterol and triglycerides were determined using enzymatic methods. LDL cholesterol level was calculated using Friedewald formula. The changes in lipid profile (LDL decrease with simultaneously HDL increase) occurred after at least 10 sessions of cryostimulation.

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### Introduction

Whole-body cryostimulation in a cryogenic chamber uses very low temperatures (ranging from  $-100$  to  $-160\text{ }^{\circ}\text{C}$ ) over a short time span (1–3 min) to induce systemic physiological responses. It is based on the heat exchange between surfaces with different temperatures [30]. Heat is absorbed by cooled surface tissues from deeper situated tissues at the rate proportional to their difference in temperature.

A desired response to a systemic effect of cryogenic temperatures (below  $-100\text{ }^{\circ}\text{C}$ ) is a hyperaemic reaction (rebound effect), stimulatory in character. Exposure in cryogenic chamber, under a supervision of a physician and in compliance to commonly accepted rules

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concerning cryostimulation, is not harmful or dangerous to healthy individuals, although there exist several contraindications. Banfi et al. reported that whole-body cryotherapy is not deleterious to cardiac function in healthy individuals [1]. Documented analgesic, anti-inflammatory and antioedematous action reduces increased muscle tension and therefore cryotherapy has been increasingly often applied in sport and medicine, in combination with additional forms of treatment [13,14,25,34,36].

It is postulated that cryostimulation mobilizes the white blood cells, particularly immunocompetent lymphocytes [4,15,21,22]. The effect on red blood cells has not been positively confirmed. Banfi et al. did not observe changes in the level of hematocrit, whereas red blood cells and mean corpuscular hemoglobin decreased after 5 cryostimulation sessions [2,3]. On the contrary, significant increase in erythrocytes count, hemoglobin concentration, hematocrit value and the mean corpuscular value (MCV) were reported by Stanek et al. [33].

It is unclear how repeated cryostimulation influences the level of pro-inflammatory and anti-inflammatory mechanisms. There is a report of increased anti-inflammatory cytokine IL-10 and decreased pro-inflammatory IL-2 and IL-8 after 5 systemic cryosti-

mulation sessions [2,3]. On the other hand, another report shows increased levels of IL-6 in response to 10 cryostimulation sessions [22]. Some authors report that cryostimulation leads to an increase in plasma ACTH and cortisol, epinephrine and norepinephrine [38], while others have not observed any stimulation of traditional stress hormones [20]. Smolander et al. reported that whole-body cryotherapy treatments in healthy females did not lead to disorders related to altered secretions of the growth hormone, prolactin, thyrotropin, or thyroid hormone [32]. Cryotherapy resulted in decreased levels of testosterone (T) and estradiol (E2) in football players, albeit with no changes in the concentration of luteinizing hormone (LH) and dehydroepiandrosterone (DHEA-S) [18]. Recent reports also suggest a stimulating antioxidative effect of cryostimulation [8,21].

There are no reports in the available literature on the influence of systemic cryostimulation on lipid levels in the human blood serum. The only information comes from experimental animal models [31]. Therefore, an attempt has been made in this study to assess the effect of a series of systemic cryogenic treatment on the lipid profile of healthy subjects.

The inconclusive results of many studies are partly due to different procedures and protocols of cryotreatment. Additionally, it has not been unequivocally determined if the positive effect of repeated stimulations depends on their number in a series. This prompted us to apply a varying number of cryostimulations (5, 10 and 20), in order to determine whether:

- (1) Cryostimulation has an effect on lipid parameters in healthy subjects and whether this method can be considered a method supporting the prevention of hyperlipidaemia.
- (2) Potential changes in lipid profile depend on the number of treatments used in a series.

## Material and methods

The study was carried out on the total of 69 physically active male volunteers, aged 20–40 years. The first group (I) consisted of male athletes (mean age  $26 \pm 2.5$  years, mean body-mass index  $27.5 \pm 2.3$  kg/m<sup>2</sup>). They were selected randomly from the Italian national rugby team (10 athletes). During the study, the subjects continued with their regular training. The workload was the same as in the previous 6 weeks. The second group (II) consisted of 39 men (mean age  $31.7 \pm 7.9$  years, mean body-mass index  $26.5 \pm 3.3$  kg/m<sup>2</sup>), among whom there were members of the police and military special units. These men were involved in daily and specialised physical training. The third group (III) consisted of 20 men (mean age  $34 \pm 5.7$  years, mean body-mass index  $27.2 \pm 2.9$  kg/m<sup>2</sup>), members of the police and military special units. Characteristics of the study groups are presented in Table 1. Tests were conducted at three centers: at the Olympic Rehabilitation Center in Spala (Poland), Małopolska Center of Cryotherapy in Cracow, Poland, and the Central Clinical Hospital of the Ministry of Interior and Administration in Warsaw. During the period of the

experiment, the participants were obliged not to change the duration and intensity of daily training, and not to make any changes in their diet. None of the participants had ever been subjected to any form of cryogenic therapy before. Each participant gave his written assent before the participation in the research, and the Local Bioethical Committee issued a formal consent, in compliance with the Declaration of Helsinki.

## Cryostimulation procedure

Before the experiment, each participant was examined by a physician in order to find any contraindications against cryostimulation.

Three different procedure of cryostimulation were applied. The first group underwent whole-body cryostimulation (WBC) once a day for 5 days (Monday, Tuesday, Wednesday, Friday, and Saturday). The second group was subjected to 10 daily cryostimulation sessions for 2 weeks, excluding Saturdays and Sundays. The third group was subject to 20 daily cryostimulations for 4 consecutive weeks, with interruptions for Saturdays and Sundays. Cryostimulation was applied every day between 7.30 and 8.30 am. The participants were exposed to sessions of extremely low temperature ( $-130$  °C) in a cryogenic chamber. Subjects entered the chamber in groups of five or four. Each session lasted 3 min. Entry to the cryochamber was preceded by a 30 s adaptation period in the vestibule at a temperature of  $-60$  °C, after which the subjects went directly to the proper chamber ( $-130$  °C). In the chamber, participants moved slowly, walking in a circle, one behind another, without mutual contact, without any additional movements and conversations. After a minute a change in direction of walking was recommended. All the time the contact with the participants was maintained with the participants via the camera and microphones. Before each treatment, systolic and diastolic blood pressure was measured in order to check for the most common contraindication of high blood pressure. Just before the entry into the cryochamber, the participants thoroughly dried their bodies to eliminate the sensation of cold. During the procedure, the subjects were dressed only in shorts, socks, gloves and a hat covering the auricles against frostbite. They also wore wooden clogs. No illnesses occurred during the study period.

## Biochemical analysis

In all investigated group venous blood samples were obtained in the morning, after overnight fast, before the first session cryostimulation, and in the following morning after the last one (in group I - after 5th, II - after 10th and in group III - after 20th day of cryostimulation session). Samples were obtained from the antecubital forearm vein, after a 10 min rest in a sitting position, using evacuated tubes (Sarstedt, Germany). The serums were isolated within 1 h from collection and stored at  $-70$  °C until assay. The serum concentrations of total cholesterol (TCH), HDL cholesterol and tri-

**Table 1**  
Characteristics of the subjects (the values are mean  $\pm$  SD, minimum and maximum).

Group	Sessions of cryostimulation			Mean value $\pm$ standard deviation (SD)		
	I	II	III	I	II	III
Height (cm)				$186 \pm 7.2$	$178 \pm 5.6$	$177 \pm 4.9$
Body mass (kg)	5	10	20	$95 \pm 9.5$	$83.6 \pm 11.6$	$86.1 \pm 10.9$
BMI (kg/m <sup>2</sup> )				$27.5 \pm 2.3$	$26.2 \pm 3.3$	$27.2 \pm 2.9$

BMI – body-mass index.

I – the group of 10 male athletes underwent once daily WBC treatment for five days.

II – the group of 39 male underwent once daily WBC treatment for ten days.

III – the group of 20 male underwent once daily WBC treatment for twenty days.

glycerides (TG) were determined with commercial kits, using enzymatic methods (Alpha Diagnostics, Poland). LDL cholesterol was calculated using the Friedewald formula, which can be used with TG values not exceeding 350 mg/dl:

$$\text{LDL [mg/dl]} = \text{total cholesterol (TCh)} - \text{HDL cholesterol} - (\text{TG}/5).$$

In addition, in groups II and III we determined the concentration of non-esterified fatty acids (NEFA) using colorimetric method and Randox kits (Randox Laboratories Ltd., UK). In addition, the following ratios were calculated TG:TGh, TCh: HDL and LDL:HDL before and after the series of cryostimulations.

Results are expressed as mean  $\pm$  S.D. Statistical analysis was performed using the Statistica 6 package. Data were checked for normal distribution using the Shapiro–Wilk test. Data were tested by one-way ANOVA. Since in some cases the distribution was not normal, when a significant *F*-value was found, a Friedman non-parametric post hoc test and a non-parametric Wilcoxon post hoc test for a dependent variable was performed. The accepted level of significance was defined at  $p < 0.05$ . Data are presented as mean values  $\pm$  SD.

## Results

The results of lipid profiles in response to different procedures of whole-body cryostimulation in a cryochamber ( $-130^\circ\text{C}$ ), for five, ten and twenty sessions, are presented in Table 2. Our results show elevated initial levels of total cholesterol and LDL in group I, which were above the desired value for each age group. HDL level was within a normal range. In the remaining groups, the obtained concentrations of lipid fractions were within the reference mean values. The control was initial values of the examined parameters within a given analyzed group, and therefore the initial differences in the lipid profile regarding LDL level likely have no effect on the final results of this study. The results of individual groups after a given number of stimulations were referenced to the values before stimulations, individually in each group.

Five sessions of whole-body cryostimulation in a cryogenic chamber did not change lipid profile of the examined subjects (group I). In group II, subjected to a series of 10 sessions, the level of TG values statistically significantly decreased from  $99.7 \pm 51.3$  to  $80.5 \pm 41.3$  mg/dL ( $p \leq 0.05$ ). The changes were more pronounced in the group III subjected to 20 sessions, where the level of TG decreased significantly from  $108 \pm 5$  to  $69.4 \pm 27$  mg/dL ( $p \leq 0.01$ ). In addition, group III showed a significant reduction in LDL levels from  $97.7 \pm 48$  to  $72.8 \pm 52$  mg/dL ( $p \leq 0.001$ ), reduction in TCh from  $172.6 \pm 44$  to  $151.8 \pm 53$  mg/dL ( $p \leq 0.05$ ), while a significant increase was observed for HDL, from  $53.2 \pm 16$  to  $63.1 \pm 27$  mg/dL.

In the experimental groups no changes were observed for glucose, both after applying a series of 10 and 20 cryostimulations. According to NEFA, in groups II no changes was observed after treat-

ment, while in the group III, which continued cryostimulation until 20th day, NEFA levels rise, reaching higher values than the initial level before stimulation ( $p \leq 0.05$ ). Comparing the ratios of individual lipid fractions, a statistically significant decrease in the TG fraction was observed in relation to total cholesterol, while HDL fraction increased in comparison with total cholesterol and LDL cholesterol after 20 cryostimulations Table 3. No changes in the proportions between lipid fractions were observed after 5 and 10 cryostimulations.

## Discussion

Despite a growing interest in cryostimulation and cryotherapy, not many experimental studies provide unambiguous data on the effects of extremely low temperatures on a healthy body and selected metabolic pathways.

The response of the body to cold occurs through changes in the endocrine, circulatory, nervous-muscular and immunological systems [10,16,19,20,23,26,28]. Acute cold stress stimulates sympatho-adrenal secretion with potent effects on energy consumption. Following  $\beta$ -adrenergic receptor activation, catecholamines stimulate lipolysis, hepatic glycogenolysis. Pancreatic secretion of glucagon is also elevated during cold exposure, promoting lipolysis [35]. It was suspected that such complex reactions should have a significant effect on lipid profile in human, but the influence of whole-body cryotherapy or cryostimulation on plasma lipids has not been extensively studied.

This is the first report about influence of whole-body cryostimulation procedure on the lipid profile in humans.

Exposure to low temperatures leads to an intensified heat production in order to maintain the balance between heat and its loss. It is known that a 3 min systemic cryostimulation at  $-130^\circ\text{C}$  leads to significant decreases in skin temperature, although individual parts of the body react differently to extremely low temperatures [5]. Cold-induced thermoregulation is associated with an increase in lipid metabolism [37].

The human body uses energy derived mainly from the conversions of carbohydrates and lipids. Adipose tissues, both white and brown (although the latter is scarce in adults), are activated during exposure to cold and generate heat which is distributed throughout the body. [29].

Although the brown adipose tissue (BAT) constitutes only about 1% of the total body mass in the adult rodent, it contributes to as much as one-third of total oxygen consumption during cold-exposure. During non-shivering thermogenesis, the release of norepinephrine from the terminal endings of sympathetic neurons leads to the mobilization of fatty acids from intracellular stores of triacylglycerides and their oxidation in the mitochondria. The heat-producing potential of BAT is expressed as a function of the uncoupling protein (UCP). The greater the UCP concentration,

**Table 2**  
Changes in lipids concentration in serum in response to different cryostimulation treatment.

Group	Before cryostimulation treatment			After cryostimulation treatment		
	I	II	III	I'	II'	III'
<i>Mean value <math>\pm</math> standard deviation (SD)</i>						
Total cholesterol (mg/dL)	210 $\pm$ 40.35	169 $\pm$ 41.5	172.6 $\pm$ 44.5	217.9 $\pm$ 41.9	159.4 $\pm$ 41	151.8 $\pm$ 53.8 <sup>III</sup>
HDL cholesterol (mg/dL)	47.9 $\pm$ 12.5	51.1 $\pm$ 17.2	53.2 $\pm$ 16.5	46.1 $\pm$ 11.5	49 $\pm$ 14.1	63.1 $\pm$ 27.4 <sup>III</sup>
LDL cholesterol (mg/dL)	140.4 $\pm$ 37.2	98.6 $\pm$ 44.9	97.7 $\pm$ 48.3	147.9 $\pm$ 42.9	94.3 $\pm$ 41.8	72.8 $\pm$ 52 <sup>III</sup>
Triglycerides (mg/dL)	110.1 $\pm$ 25.7	99.7 $\pm$ 51.3	108 $\pm$ 50	120.1 $\pm$ 30.5	80.5 $\pm$ 41.3 <sup>II</sup>	69.4 $\pm$ 27.2 <sup>III</sup>
NEFA (mmol/L)	–	0.62 $\pm$ 0.3	0.64 $\pm$ 0.4	–	0.63 $\pm$ 0.4	0.79 $\pm$ 0.3 <sup>III</sup>
GLU (mg/dL)	–	85.6 $\pm$ 10.1	86.5 $\pm$ 11.6	–	88 $\pm$ 14.8	91.4 $\pm$ 17.8

<sup>\*</sup> Statistically significant difference at  $p \leq 0.05$ .

<sup>\*\*</sup> Statistically significant difference at  $p \leq 0.01$ .

**Table 3**

The ratios TG:TCh, TCh:HDL and LDL:HDL before and after the series of cryostimulation, respectively 5 in the group I, 10 in the group II and 20 in the group III.

Group	Before cryostimulation			After cryostimulation		
	I	II	III	I'	II'	III'
<i>Mean value ± standard deviation (SD)</i>						
TG:TCh	0.53 ± 0.1	0.6 ± 0.4	0.7 ± 0.3	0.56 ± 0.1	0.5 ± 0.2	0.51 ± 0.2 <sup>***</sup>
TCh:HDL	4.9 ± 2.1	3.5 ± 1.5	3.6 ± 1.2	5.1 ± 2.3	3.5 ± 1.5	2.6 ± 1 <sup>***</sup>
LDL:HDL	3.2 ± 1.8	2.1 ± 1.4	2.2 ± 1.1	3.5 ± 2	2.0 ± 1.3	1.3 ± 1 <sup>* III</sup>

\* Statistically significant difference at  $p \leq 0.05$ .

\*\* Statistically significant difference at  $p \leq 0.01$ .

the greater the capacity to uncouple mitochondrial oxidative phosphorylation so that heat is produced [9].

Energy expenditure, associated with the additional heat production, affects cholesterol metabolism in the body. Analyzing the changes in the concentrations of individual lipids in groups exposed to cryogenic temperatures, significant differences were found in the effects of cryostimulation in relation to the number of sessions. It seems that the use of 5 treatments does not affect the lipid profile of the examined individuals, while a series of 10 stimulations (a standard number recommended in various diseases and biological regeneration) induces a significant reduction in triglycerides.

The extension of cryostimulation to 20 sessions significantly alters the lipid profile in healthy subjects, reducing TCh, LDL and TG, and increasing HDL. Changes in the concentrations of these fractions were also accompanied by positive changes in their mutual proportions, but only in the group III, i.e. subjected to a series of 20 treatments. In the available literature only one publication analyzes changes in the concentrations of individual lipid fractions after exposure to cryogenic temperatures [31]. In rats exposed to  $-60$  and  $-90$  °C for 5 or 10 days, HDL and LDL cholesterol fraction decreased, and total cholesterol concentrations in animals subjected to  $-60$  °C sessions for 10 days remained unchanged. The authors also observed an increase in triglycerides in the blood serum in animals subjected to cryostimulation compared with control. A decrease in HDL cholesterol in rats after cryostimulation can be explained by the fact that HDL is the main fraction transporting cholesterol in rats, while in humans most cholesterol is found in low-density lipoproteins.

HDL and LDL are very important plasma lipoproteins that are involved in lipid metabolism and the exchange of cholesterol, cholesterol ester and triglycerides between tissues [24]. Numerous population studies have shown an inverse correlation between serum HDL levels and risk of cardiovascular disease. Function of HDL cholesterol is to enhance reverse cholesterol transport by scavenging excess cholesterol from peripheral tissues followed by esterification and delivering it to the liver and steroidogenic organs. Additionally HDL cholesterol regulates the exchange of protein and lipids between lipoproteins, and inhibit oxidation of LDL because of its antioxidant property [7]. Lipid ratio, like total cholesterol/HDL cholesterol and the LDL cholesterol/HDL cholesterol ratio correlate with cardiovascular disease. A significant reduction in the proportion of LDL cholesterol to HDL cholesterol and of total cholesterol to HDL cholesterol seems beneficial enough to consider whole-body cryostimulation a useful method of atherosclerosis prevention. HDL, the beneficial form of blood cholesterol, protects the artery wall against atherosclerosis and cardiovascular disease [11,12].

The anti-atherosclerotic influence of HDL cholesterol is complex, including reverse cholesterol transport, tissue which lowers cholesterol levels. HDL fraction captures excess cholesterol from peripheral cells and allows its transport to the liver [6].

Additionally, HDL accumulates high levels of lipid hydroperoxides, suggesting that it may be a carrier for oxidized species released during the degradation of triglyceride-rich lipoproteins [17,27].

The concentration of free fatty acids is the result of their release from adipose tissue during lipolysis and TG plasma, caused by the influence of lipoprotein lipase on chylomicrons and VLDL and the offtake by cells, mainly working muscles. A single determination of FFA concentration is a poor diagnostic because of its large variability, but it still seems valuable to estimate these concentrations in response to various stimuli, including cryogenic temperatures. Only on the basis of changes in the FFA concentrations, may one draw conclusions on the activity of lipoprotein lipase and triacylglycerol lipase in adipocytes (this study was performed on an empty stomach, i.e. during a period when this lipase is not inhibited by insulin).

The observed changes in the lipid profile induced by cryostimulation are quite promising. However, one should remember about the limitations of this study which could be taken under consideration during conclusion about the lesser effectiveness of 10 stimulations and no effectiveness of 5 stimulations. Based on the obtained data, one cannot exclude the probability that changes in level of lipoproteins could occur after some time of cryogenic treatment (a delayed-action response), as adaptation changes connected with the regulation of enzymatic activity at the level of biosynthesis. Given this, changes in the lipid profile could have occurred even after 5 and 10 stimulations, but the effect could only be observable 2 or 3 weeks after the completion of the series. One could then argue that the first several stimulations had some effect on the changes in the level of lipoproteins and the continuation of stimulations changed nothing. Although this interpretation seems to be much less probable, it cannot be excluded altogether. It should be examined by further research on cryostimulation with respect to adaptation changes and the long-term effects of cryogenic temperatures during whole-body cryostimulation.

## Conclusion

The whole-body exposure to cryogenic temperatures in a cryochamber leads to changes and beneficial effect in lipids profile in healthy human. The cryostimulation could be a physical treatment used in hyperlipidaemia prevention. The minimum number of once-daily sessions with a positive effect is 10, and the optimum number is 20 once-daily sessions.

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