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ORIGINAL ARTICLE

The effect of prolonged whole-body cryostimulation treatment with different amounts of sessions on chosen pro- and anti-inflammatory cytokines levels in healthy men

ANNA LUBKOWSKA[1,2], ZBIGNIEW SZYGUŁA[3], DARIUSZ CHLUBEK[2] & GIUSEPPE BANFI[4,5]

[1]*Department of Physiology, Faculty of Natural Sciences, Szczecin University, Szczecin, Poland,* [2]*Chair and Department of Biochemistry and Medical Chemistry, Pomeranian Medical University, Szczecin, Poland,* [3]*Institute of Human Physiology, University School of Physical Education, Krakow, Poland,* [4]*School of Medicine, University of Milan, Italy, and* [5]*IRCCS Galeazzi, Milan, Italy*

Abstract

Cryotherapy is used in the early treatment of acute injuries (sprains, strains, fractures) yet only a few papers discuss the possible influence of whole-body cryostimulation on inflammation mechanisms or immunology. It is postulated that cold exposure can have an immunostimulating effect related to enhanced noradrenaline response and can be connected with paracrine effects. The aim of this study was to examine the effect of different sequences of whole-body cryostimulations on the level of pro- and anti-inflammatory cytokines in healthy individuals. The research involved 45 healthy men divided into three groups. The groups were subjected to 5, 10 or 20, 3-minute long whole-body cryostimulations each day at −130°C. Blood was collected for analysis before the stimulations, after completion of the whole series, and 2 weeks after completion of the series, for the examination of any long-term effect. The analysis of results showed that in response to cryostimulation, the level of ani-inflammatory cytokines IL-6 and IL-10 increased while Il-1α cytokine level decreased. It seems that the most advantageous sequence was the series of 20 cryostimulations due to the longest lasting effects of stimulation after the completion of the whole series of treatments.

Key Words: *Whole-body cryostimulation, proinflammatory cytokine, anti-inflammatory cytokine*

Introduction

One of the advantages of whole-body cryostimulation in medicine, rehabilitation, sport and biological renewal is the provocation of systemic physiological responses that lead to a reduction in inflammatory reaction [1]. However, reports on the effects of cryogenic temperatures are often contradictory, mainly due to the differing number of treatments applied during the tests, differing durations of a single treatment, participation of people with various diseases in the study, and the lack of information about changes induced by cryogenic temperature in healthy patients [2–4].

Usually cryostimulations are performed at temperatures from −110°C to −160°C, depending on the available cryochamber. The most frequently used temperature is −130°C, relatively well tolerated by patients; the stimulation may then take from 2.5–3 minutes without complications, e.g. frostbite. Cryostimulation is usually performed once a day for 10 following days, but there is no sufficient information and research showing that this number is the most advantageous. In our previous studies, the most beneficial effects on the lipid profile induced by cryostimulation were observed after the application of 20 daily 3-min treatments [5]. Numerous studies confirm that short-term whole-body cold exposure induces an oxidative stress but does not decrease the antioxidant capacity [6–10]. Additionally it is known that hypothermia inhibits the expression of

Correspondence: Anna Lubkowska PhD, Department of Physiology, Faculty of Natural Sciences of Szczecin University, Ul. Felczaka 3c, 71-412 Szczecin, Poland. Tel: +48 091 4441596. E-mail: annalubkowska@gmail.com

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inflammatory mediators and induces expression of anti-inflammatory cytokines [11–13].

Cold is known to affect leukocyte mobilization, and it is suggested that cold exposure initiates changes in cytokine expression associated with a nonspecific acute phase reaction that could be the affect of multiple interactions between the cytokines and neuroendocrine hormones [14]. Despite a recently growing interest in cryostimulation, relatively little is known about the physiological modulation of the immune system, cytokine expression and their serum concentration by cryogenic temperatures, both as cryostimulation and as a cryotherapy. It seems important to know the influence of repeated exposure to the stress induced by cryogenic temperatures affecting the whole-body of healthy people in order to use this knowledge efficiently in clinical practice. Therefore, the aim of this study was to observe changes in the levels of cytokines under the influence of repeated systemic cryostimulation in young healthy men. In addition, it was examined whether there were differences in response to cryostimulation, depending on the daily number of applied treatments.

Material and methods

The research involved 45 healthy men, aged 22 ± 0.8 years, with normal body-weight, who had never been subjected to any form of cryotherapy. Each participant gave his written consent before participation in the research, and the Bioethical Committee of the Regional Medical Society issued their formal consent, according to the Declaration of Helsinki.

Prior to the start of the experiment, each participant was examined by a physician to test for any contraindications against cryostimulation. Before each treatment, systolic and diastolic blood pressure was measured in order to check for the most common contraindication of high blood pressure. The participants were non-smokers and they were advised to maintain the same level of physical activity and their regular diet during the period of tests. The participants were randomized into three groups with the same numerical amount. Three different procedures of cryostimulation were used for the appropriate group of men during the study. The first group (A, $n = 15$) underwent whole body cryostimulation (WBC) once a day for 5 consecutive days. The second group (B, $n = 15$) was subjected to 10 daily cryostimulation sessions for 2 weeks (10 sessions), excluding Saturdays and Sundays. The third (C, $n = 15$) group was subjected to 20 daily cryostimulations for 4 consecutive weeks, with interruptions for Saturdays and Sundays. The cryostimulations took place every day at the same time, between 8 a.m. and 10 a.m.

Each session of whole-body cryostimulation lasted 3 minutes. Entry to the cryo-chamber was preceded by a 30-second adaptation period in the vestibule at a temperature of − 60°C, after which the subjects went directly to the chamber proper (−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 1 minute a change in the direction of walking was recommended. All the time contact with the participants was maintained via a camera and microphones. Just before 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. In order to protect the upper airways, noses and mouths were secured with a surgical mask. No illnesses occurred during the study period.

Biochemical analysis

In all the groups, blood samples were obtained from an antecubital forearm vein using vacutainer system tubes (Sarstedt, Germany), after overnight fasting, in the morning before the treatment and after a 10-minute rest in a sitting position.

The analyses of the levels of pro- and anti-inflammatory cytokines were performed on blood samples taken four times from each group, on the following days of the experiment:

(1) Morning, before any cryostimulation, in all the examined groups (1A; 1B; 1C).
(2) Morning, on the day following the last cryostimulation for the given group (2A – after five treatments in group A; 2B – after 10 treatments in group B; 2C – after 10 treatments in group C; 3C – after 20 treatments in group C).
(3) Two weeks after the completion of 5,10 and 20 treatments in respective groups (3A; 3B; 4C).
(4) Control sampling in groups A and B, in order to examine the long-term effects of cryostimulation, on the day of the last blood sampling in group C.

Blood samples for morphological examination were collected to vacutainer tubes (2.4 ml) containing anticoagulant (EDTA). For the determination of serum cytokines, blood was collected into vacutainer tubes (7.5 ml) containing no anticoagulant. It was incubated at room temperature for approximately 30 min to allow clotting and then immediately centrifuged (1200 g/for 10 min) at +4°C. Directly after centrifugation, serum samples were divided into aliquots and immediately deep-frozen at −70° C until the time of analysis.

In samples no.1 hematological parameters were determined, including number of erythrocytes (RBC),

hemoglobin concentration (Hb), the hematocrit value (Hct), number of leukocytes (WBC) and thrombocytes (PLT). Cytokines: IL-1α, IL-1β, IL-6, IL-10, IL-12 and TNFα were determined in serum using enzyme-linked immunosorbent assay for quantitative detection of human interleukin (Human Interleukin Elisa, Bender MedSystems GmbH, Vienna, Austria). Method sensitivity and intra-assay and inter-assay coefficient of variation was: for IL-1α: 1.1 pg/ml, <5.4%, <10%; IL-1β: 0.3 pg/ml, <5.1%, <8.6%; IL-6:0.92 pg/ml, <3.4%, <5.2%; IL-10: 1.0 pg/ml, <3.2%, <5.6%; IL-12: 2.1pg/ml, <3.0%, <4.8%; TNFα: 0.13 pg/ml, <8.5%, 9.8%.

Statistical analysis

Statistical analysis was performed using the Statistica 6 package. We assessed the distribution of the analysed variables using a Shapiro-Wilk test. The results showed that in some cases the distributions deviated from normal distribution, so a detailed statistical analysis using nonparametric tests was necessary. When a significant F-value in Friedmans' analysis was found a Wilcoxon post-hoc test for dependent variables was performed. For the parameters with normal distribution the results are expressed as the mean value with standard deviation (± SD), in other cases the results are expressed as median, the value of the lower quartile (Q_{25}) and the value of the upper quartile (Q_{75}). The level of statistical significance was $p < 0.05$. In order to demonstrate whether the observed correlations were statistically significant, we used the Spearman's rank correlation coefficient.

Results

The examined groups of men were homogeneous with regard to age, weight and body height, and BMI. In all patients blood morphology values were within the range of reference values for respective age groups. Anthropometric characteristics and blood morphology are presented in Table I. The levels of cytokines IL1-α, IL1-β, IL-6, IL-10, IL-12, TNFα in serum, before and after cryostimulation are shown in Table II. There were no statistically significant differences in the initial levels of any of the studied cytokines between the examined groups. These values were low and typical for healthy persons. The results obtained in subsequent samples were referred to the initial level for the group, treated as the control. After five daily treatments using whole-body cryostimulation in group A, a statistically significant increase was observed in IL-6 levels ($\Delta = 0.12$ pg/ml) and IL-10 ($\Delta = 0.35$ pg/ml, $p \leq 0.05$) compared to the initial values. In this group, 2 weeks after exposure the increased IL-10 levels continued while the levels of IL-6 returned to the initial state. Similarly, after applying the series of 10 treatments in groups B and C, the levels of these cytokines increased; most IL-6 ($\Delta = 0.15$ pg/ml, $p \leq 0.01$), slightly less significant was the increase in IL-10 ($\Delta = 0.1$ pg/ml, $p \leq 0.05$). Two weeks after the series of 10 treatments in group B these values returned to the initial state, in both cases. It was different in group C which continued for 20 cryostimulations. After completion of the series, the levels of the aforementioned interleukins were still elevated compared to the initial values (ΔIL-6 = 0.16 pg/ml, $p \leq 0.01$; ΔIL-10 = 0.08 pg/ml, $p \leq 0.05$). Two weeks after completion of the series of 20 cryostimulations, cytokine levels in serum returned to the initial values. The opposite direction of changes was observed for IL1α. As early as after the series of five treatments in group A, the level of this cytokine significantly decreased ($\Delta = 0.09$ pg/ml, $p \leq 0.05$). In group B, after 10 treatments, this decrease was $\Delta = 0.17$ pg/ml and in group C it was $\Delta = 0.25$ pg/ml ($p \leq 0.05$). In group C which continued treatment, a further reduction in the level of IL-1α was observed: $\Delta = 0.32$ pg/ml ($p \leq 0.05$), after a series of 20 treatments. Two weeks after the series of both 5 and 10 treatments, the levels returned to the initial values, but remained decreased in group C 2 weeks after the series of 20 treatments.

The use of whole-body cryostimulation, regardless of the number of treatments in the series, did not result in changes in the levels of IL-1β, IL-12 or TNFα.

No significant correlations were found between body weight, BMI and initial levels of cytokines. However, increases in the levels of IL-6 and IL-12, both after 10 and 20 treatments, significantly correlated with the BMI of the subjects (Table III).

Discussion

Whole-body cryotherapy (WBC) is recommended for patients suffering from arthritis, osteoarthritis, fibromyalgia, acute injury, trauma, chronic pain, and

Table I. Characteristics of the examined groups (the values are mean ± standard deviation (SD), minimum and maximum).

	Mean value ± SD		
Group	A	B	C
Height (m)	1.82 ± 0.04	1.79 ± 0.06	1.81 ± 0.04
Body mass (kg)	74.8 ± 3.7	73.5 ± 5.4	77.6 ± 9.4
BMI (kg/m^2)	23.2 ± 1.4	23.4 ± 1.8	23.4 ± 2.1
WBC (10^9/L)	6.9 ± 2.0	6.5 ± 1.5	7.0 ± 1.3
RBC (10^{12}/L)	5.2 ± 0.3	5.1 ± 0.2	5.3 ± 0.1
HGB (g/L)	13.2 ± 1.0	13.3 ± 0.6	12.9 ± 1.3
HCT (%)	44 ± 3	46 ± 2	44 ± 3
PLT (10^9/L)	234 ± 43	223 ± 32	257 ± 40

A – the group underwent once-daily WBC treatment for five sessions; B – the group underwent once-daily WBC treatment for 10 sessions; C – the group underwent once-daily WBC treatment for 20 sessions; BMI, body mass index; WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; PLT, thrombocytes.

Table II. Changes in the level of interleukin in serum following the different amount of sessions of cryostimulation treatment.

Group and duration of the samples collection		Median and the value of the lower and the upper quartile(Q_{25}–Q_{75})					
		IL1-α (pg/ml)	IL1-β (pg/ml)	IL6 (pg/ml)	IL10 (pg/ml)	IL12 (pg/ml)	TNFα (pg/ml)
A	1A	0.93	2.27	1.62	0.72	2.2	22.6
		(0.87–0.98)	(2.00–2.80)	(1.10–1.76)	(0.68–0.75)	(1.8–2.5)	(19.0–25.3)
	2A	0.84^{*1A}	2.12	1.74^{**1A}	1.07^{*1A}	2.1	23.0
	(after 5 sessions)	(0.83–0.88)	(1.95–2.30)	(1.70– 1.94)	(0.78–2.00)	(1.9–2.7)	(21.0–24.1)
	3A	0.90	2.48	1.60	0.79^{*1A}	2.3	21.0
	(2 weeks after 5 sessions)	(0.82–0.92)	(1.84–2.56)	(1.14–1.78)	(0.69–0.84)	(2.1–2.8)	(20.3–28.8)
	4A	0.88	2.33	1.50	0.77	2.4	20.0
	(control for 4C)	(0.78–1.00)	(2.13–2.47)	(1.42–1.62)	(0.68–0.82)	(2.2–2.5)	(18.4–25.7)
B	1B	1.00	2.35	1.51	0.64	2.0	22.0
		(0.94–1.46)	(1.98–2.60)	(1.27–1.54)	(0.62–0.66)	(1.8–2.6)	(18.8–24.1)
	2B	0.83^{*1B}	2.12	1.66^{**1B}	0.73^{*1B}	2.3	21.1
	(after 10 sessions)	(0.81–0.92)	(1.94–2.80)	(1.56–1.73)	(0.68–0.77)	(2.1–2.6)	(19.3–22.1)
	3B	0.89	2.10	1.54	0.66	2.5	19.4
	(2 weeks after 10 sessions)	(0.80–1.00)	(1.92–2.30)	(1.35–1.87)	(0.64–0.72)	(2.0–3.0)	(18.3–21.8)
	4B	0.89	2.31	1.56	0.65	2.4	22.2
	(control for 4C)	(0.77–1.36)	(1.77–2.85)	(1.33–1.71)	(0.61–0.68)	(2.1–3.1)	(19.7–23.1)
C	1C	1.20	2.30	1.56	0.71	2.3	22.4
		(1.00–1.60)	(2.00–2.56)	(1.28–2.16)	(0.70–0.80)	(2.1–3.1)	(21.0–23.2)
	2C	0.95^{*1C}	2.30	1.70^{**1C}	0.81^{*1C}	2.4	24.0
	(after 10 sessions)	(0.89–0.99)	(2.00–3.10)	(1.38–2.26)	(0.79–0.89)	(2.0–2.8)	(22.1–25.3)
	3C	0.88^{*1C}	2.03	1.72^{**1C}	0.79^{*1C}	2.0	24.3
	(after 20 sessions)	(0.83–0.92)	(1.83–2.40)	(1.40–1.90)	(0.72–0.80)	(1.8–2.8)	(23.0–26.1)
	4C	0.92^{*1C}	2.32	1.58	0.76	2.4	21.5
	(2 weeks after 20 sessions)	(0.84–1.00)	(1.95–2.50)	(1.46–1.68)	(0.71–1.00)	(2.1–3.2)	(20.0–22.6)

Statistically significant difference at $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$.

muscle spasms [1,2,15] and is widespread in the biological regeneration and rehabilitation of athletes to improve recovery from muscle injuries [4,16]. Recently we have observed a growing interest in cryostimulation as a method of prevention and treatment of obesity. Most of the aforementioned disorders are accompanied by acute or chronic, clinical or subclinical inflammation. At the same time still very little is known about the modulation of the human immune system, inflammatory mediator response, and cytokine expression and its serum or plasma levels by cryogenic temperatures.

The inflammatory reaction involves cells (migration, adhesion, diapedesis, chemotaxis) and humoral and immune responses associated with the release of C-reactive protein, complement proteins, interleukins, interferon and the synthesis of antibodies [17]. Cytokines, small-molecule proteins with autocrine, paracrine and endocrine action, are involved in the regulation of cell migration, inflammation, proliferation, hematopoiesis, lipolysis, and glucose homeostasis [18–21]. A dynamic balance exists between the proinflammatory cytokines and the anti-inflammatory components of the human immune system, and additionally almost all the anti-inflammatory cytokines, with the exception of a receptor (IL-1ra), have at least some proinflammatory properties [22]. Production of various pro-and and anti-inflammatory cytokines is upregulated rapidly in response to different forms of stress, e.g. exercise [23–27]. In this paper we analysed changes in the level of pro-inflammatory and anti-inflammatory cytokines: IL-1α, IL-1β, TNF-α IL-12, IL-10 and the most important in immunological and cell regulation IL-6, which, although initially considered a pro-inflammatory mediator, is currently recognized mainly as an anti-inflammatory agent [28,29]. In this study it was found that stress induced by exposure to extremely low temperatures causes changes in the level of cytokines in healthy individuals. Particularly interesting is the fact that the increased levels of cytokines involved all the anti-inflammatory ones, most significantly IL-6.

This increase occurs even after the application of only five daily 3-min-long cryostimulations. In our previous studies, we observed increased levels of white blood cells in response to a series of 10 treatments and at the same time we showed that even a single, 3-min-long whole body exposure to cryogenic temperature (− 130°C) leads to increased levels of interleukin-6 [30]. This is confirmed in this study. Because during cryostimulations an increase in circulating IL-6 is accompanied only by an increase in anti-inflammatory interleukin IL-10, without an increase in classical pro-inflammatory cytokines (IL-1α, IL-1β and TNF α) the role of IL-6 in this case could be deemed anti-inflammatory. These dependencies resemble those induced by physical effort. It has been noticed that the anti-inflammatory effect of acute exercise displays as an increase in the level of circulating IL-6 with following IL-1ra and IL-10 rise [29]. It indicates that the activation of cytokine

Table III. Correlation (Spearman rank correlation coefficient) between body mass index and cytokine level at the beginning of the study and after different amounts of cryostimulation sessions.

		BMI
IL1-α	Before the treatment	0.21
	After 5 sessions	0.10
	After 10 sessions	0.06
	After 20 sessions	0.08
IL1-β	Before the treatment	−0.02
	After 5 sessions	−0.16
	After 10 sessions	−0.21
	After 20 sessions	−0.31
IL6	Before the treatment	0.10
	After 5 sessions	0.30
	After 10 sessions	0.80*
	After 20 sessions	0.60*
IL10	Before the treatment	−0.10
	After 5 sessions	0.27
	After 10 sessions	0.09
	After 20 sessions	0.07
IL12	Before the treatment	0.36
	After 5 sessions	0.38
	After 10 sessions	0.68*
	After 20 sessions	0.64*
TNFα	Before the treatment	0.09
	After 5 sessions	0.27
	After 10 sessions	0.25
	After 20 sessions	0.30

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

cascade after exposure to cryogenic temperatures, for example, during exercise is caused by a mechanism different to that during infection.

The accompanying reduction in the level of a proinflammatory interleukin IL-1α confirms the positive effect of this form of physical treatment and shows advantages of repeated exposure of the human body to low-level stress.

The observed increase in IL-10 may be a consequence of the increased secretion of IL-6. *In vivo* studies show that the administration recombinant interleukin-6 increases plasma IL-10, which in turn inhibits the release of both proinflammatory cytokines IL-1α, IL-1β, TNFα [31] and chemokines, including IL-8 and macrophage inflammatory protein α (MIP α) from lipopolysaccharides (LPS)-activated human monocytes [32]. Additionally, anti-inflammatory effects of IL-6 are demonstrated by the stimulation of the production of IL-1ra (IL-1 receptor antagonist), the release of soluble TNFα receptors, and down-regulation of the synthesis of IL-1 and TNFα [22,26,30]. Our research confirms earlier reports about no changes in pro-inflammatory interleukins IL-1β and TNFα after cryostimulation [3,15].

Our observations of immunostimulation and the protective action of cryostimulation are consistent with other reports. Banfi et al. [13] shows that whole body cryotherapy leads to an increase in anti-inflammatory interleukin-10 and a decrease in pro-inflammatory interleukin-2 and IL-8. Additionally, this author observed a decrease in sICAM-1 (intercellular adhesion molecule 1) and prostaglandin E2 which intensify the anti-inflammatory response after cryostimulation [16]. It is also known that inflammation leads to an increase in the level of pro- and anti-inflammatory cytokines. A question arises if such low temperatures as a stressogenic factor exacerbate inflammation in the body. It seems that this hypothesis can be rejected, as in such a case the increase would be chronic and significantly higher (2–3 times) [18,26].

IL-6 is produced by monocytes and macrophages, fibroblasts, T and B cells, endothelial cells, adipocytes and in the contracting skeletal muscle. A number of studies demonstrated that following exercise, the basal plasma IL-6 concentration may increase considerably, and the response is sensitive to exercise intensity and the muscle mass involved in the contractile activity [18,24,33]. Initially it was thought that an exercise-induced increase in IL-6 was a consequence of an immune response to local damage in the working muscles but nowadays it is clear that the contracting skeletal muscle per se is the main source of the IL-6 in the circulation in response to physical effort [25,26], and therefore IL-6 can be classified as a myokine [34,35].

Sudden exposure to cold induces physiological responses of the sympathetic nervous system, leading to minimized heat loss and the simultaneous increase in heat production (peripheral vascular spasm, and shivering and non-shivering thermogenesis) [36]. It can therefore be assumed that one of the possible causes of the increase in the aforementioned myokine during cryostimulation is the shivering thermogenesis during cryostimulation, based on involuntary repetitive rhythmic contractions of skeletal muscles. Shivering thermogenesis is activated in the first minutes of exposure to cold, initially in the muscles of the torso and limbs, and is assisted by the secretion of catecholamines [37]. The skeletal muscles do not have to be the only source of elevated IL-6. The adipose tissue may also contribute markedly to IL-6 increase in the circulation even during rest [2]. Additionally IL-6 mRNA levels increase in adipose tissue during exercise [38].

Some reports suggest that approximately 30% of circulating IL-6 comes from adipose tissue and that visceral adipose tissue secretes more IL-6 than subcutaneous adipose tissue [39]. It is recognized that obesity results in the secretion of TNFα, IL-1β and IL-6, and these cytokines are secreted both from adipocytes and macrophages within the adipose tissue bed [2].

There have been some reports about the relationship between BMI and the level of C-reactive proteins (CRP), and between BMI and IL-6 [32,40,41]. In our study, we checked if there were relationships between body weight, BMI and the level of the examined cytokines. The results were very interesting. When examining the initial values and after the series

of five treatments, there were no such correlations, but the series of 10 and 20 cryostimulations resulted in the emergence of significant and positive correlations between the levels of IL-6 and IL-12 and BMI. The correlation coefficients between IL-6 and BMI were respectively: r = 0.8 after 10 treatments and r = 0.6 after 20 sessions, correlation between IL-12 and BMI: r = 0.68 and r = 0.64 after 20 stimulations (Table III). This may indicate the role of adipose tissue in the synthesis of these cytokines during exposure to cold. Our earlier studies on the effects of whole-body cryostimulation on lipid profile in healthy subjects have shown that the use of only 10 and most preferably 20 treatments produces beneficial changes in lipid fractions, which were not observed after five treatments [5]. Il-6 is identified as a modulator of fat metabolisms in humans, increasing lypolysis and fat oxidation without causing hypertriacylglycerolemia [42]. As observed in this study, the beneficial effect of cryostimulation – the increased levels of anti-inflammatory cytokines – continued only during the series of cryostimulations, but was not visible 2 weeks after the series of treatments, regardless of their number. The effect of reduced proinflammatory IL-1α, observed during the series of 5, 10 or 20 treatments, was only observed after the end of the series of 20 treatments.

In order to observe the probable delayed effect of cryostimulation, in group A (five treatments) blood was taken after a period of 5 weeks, and in group B (10 treatments) after 4 weeks from the last treatment. At the same time blood was taken 2 weeks after the end of 20 treatments in Group C. Cytokine levels observed in groups A and B nullified assumptions about the long-term delayed effect of cryostimulation. Therefore in accordance with earlier observations, it seems that the series of 20 daily 3-min-long cryostimulations is more advantageous than the routinely used series of 10 treatments.

Conclusion

Present and previous studies have confirmed the influence of whole-body cryostimulation on the level of circulating pro-and anti-inflammatory cytokines. Changes in the cytokine level caused by cryogenic temperatures seem to stimulate immune mechanisms in the body. It is worth noting that the changes depend on the duration of the stimulation series. Although the most common procedure is limited to 10 cryostimulations, our results suggest the use of 20 stimulations in order to induce adaptation changes. Taking into account changes in the lipid profile and the cytokine level in the examined individuals, we think it is worth considering this form of physiotherapy as a potential method supporting prevention of diseases with chronic, subclinical inflammation, including the metabolic syndrome and obesity.

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References

[1] Nadler SF, Weigang K, Kruse RJ. The physiologic basis and clinical applications of cryotherapy and thermotherapy for the pain practitioner. Pain Physician 2004;7:395–9.
[2] Swenson C, Sward L, Karlsson J. Cryotherapy in sports medicine. Scand J Med Sci Sports 1996;6:193–200.
[3] Straburzy ska-Lupa A, Konarska A, Nowak A, Straburzy ska-Migaj E, Konarski J, Kijewski K, Pilaczy ska-Szcze niak Ł. Effect of whole-body cryotherapy on selected blood chemistry parameters in professional field jockey players. Polish J Physiother 2007;7:15–20.
[4] Banfi G, Lombardi G, Colombini A, Melegati G. Whole-body cryotherapy in athletes. Sports Med 2010;40:509–17.
[5] Lubkowska A, Banfi G, Dołęgowska B, d'Eril GV, Łuczak J, Barassi A. Changes in lipid profile in response to three different protocols of whole-body cryostimulation treatments. Cryobiology 2010;1:22–6.
[6] Siems W, Brenke R. Changes in the glutathione system of erythrocytes due to enhanced formation of oxygen free radicals during short-term whole body cold stimulus. Arctic Med Res 1992;51:3–9.
[7] Siems W, van Kuijk F, Maass R, Brenke R. Uric acid and glutathione levels during short-term whole body cold exposure. Free Radic Biol Med 1994;16:299–305.
[8] Dugue B, Smolander J, Westerlund T, Oksa J, Nieminen R, Moilanen R, Mikkelsson M. Acute and long-term effects of winter swimming and whole-body cryotherapy on plasma antioxidative capacity in healthy women. Scand J Clin Lab Invest 2005;65:395–402.
[9] Lubkowska A, Chudecka M, Klimek A, Szyguła Z, Fr czek B. Acute effect of a single whole-body cryostimulation on prooxidant-antioxidant balance in blood of healthy, young men. J Thermal Biol 2008;33:464–7.
[10] Lubkowska A, Doł gowska B, Szygula Z, Klimek A. The activity of selected enzymes in erythrocytes and the level of plasma antioxidants in response to single whole-body cryostimulation in humans. Scand J Clin Lab Invest 2009; 69:387–94.
[11] Scumpia PO, Sarcia JP, Kelly KM, DeMarco VG, Skimming JW. Hypothermia induces anti-inflammatory cytokines and inhibits nitric oxide and myeloperoxidase-mediated damage in the hearts of endotoxemic rats. Chest 2004; 125:1483–91.
[12] Hanusch C, Nowak K, Gill IS, Torlitz P, Rafat N, Mueller AM, Van Ackern KC, Yard B, Beck GC. Hypothermic preservation of lung allograft inhibits cytokine-induced chemoattractant-1, endothelial leukocyte adhesion molecule, vascular cell adhesion molecule-1 and intracellular adhesion molecule-1 expression. Clin Exp Immunol 2007;149:364–71.
[13] Banfi G, Melegati G, Barassi A, Dogliotti G, d'Eril G, Dugué B, Corsi MM. Effects of whole-body cryotherapy on serum mediators of inflammation and serum muscle enzymes in athletes. J Therm Biol 2009;34:55–9.
[14] Rhind SG, Castellani JW, Brenner IK, Shephard RJ, Zamecnik J, Montani SJ, Young AJ, Shek PN. Intracellular monocyte and serum cytokine expression is modulated by exhausting exercise and cold exposure. Am J Physiol Regul Integr Comp Physiol 2001;291:R66–75.

[15] Leppaluoto J, Westerlund T, Huttunen P, Oksa J, Smolander J, Dugude B, Mikkelsson M. Effects of long-term whole-body cold exposures on plasma concentrations of ACTH, beta-endorphin, cortisol, catecholamines and cytokines in healthy females. Scand J Clin Lab Invest 2008;68:145–53.
[16] Banfi G, Melegati G, Barassi A, Lombardi G, Colombini A, d'Eril G. Effects of the whole-body cryotherapy on NTproBNP, hsCRP and troponin I in athletes. J Sci Med Sport 2009;12:609–10.
[17] Całkosiński I, Dobrzyński M, Całkosińska M, Seweryn E, Bronowicka-Szydełko A, Dzierzba K, Ceremuga I, Gamian A. Characterization of an inflammatory response. Postepy Hig Med Dosw 2009;63:395–408.
[18] Fischer CP. Interleukin-6 in acute exercise and training: what is the biological relevance? Exerc Immunol Rev 2006;12:6–33.
[19] Han W, Yu Y, Liu XY. Local signals in stem cell-based bone marrow regeneration. Cell Res 2006;16:189–95.
[20] Schottenfeld D, Beebe-Dimmer J. Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. CA Cancer J Clin 2006;56:69–83.
[21] Stanley AC, Lacy P. Pathways for cytokine secretion. Physiology 2010;25:218–29.
[22] Opal SM, DePalo VA. Anti-inflammatory Cytokines. Chest 2000;117:1162–72.
[23] Pedersen BK, Akerström TC, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. J Appl Physiol 2007;103:1093–8.
[24] Pedersen BK, Fischer CP. Physiological roles of muscle-derived interleukin-6 in response to exercise. Curr Opin Clin Nutr Metab Care 2007;10:265–71.
[25] Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiol Rev 2008;88: 1379–406.
[26] Mathur N, Pedersen BK. Exercise as a mean to control low-grade systemic inflammation. Mediators Inflamm 2008;109502:1–6.
[27] Pedersen BK. The diseasome of physical inactivity – and the role of myokines in muscle–fat cross talk. J Physiol 2009; 587:5559–68.
[28] Steensberg A, Fischer CP, Keller C, Moller K, Pedersen BK. IL-6 enhance plasma IL-1ra, IL-10 and cortisol in humans. Am J Physiol Endocrinol Metab 2003;285:433–7.
[29] Petersen AM, Pedersen BK. The role of IL-6 in mediating the anti-inflammatory effects of exercise. J Physiol Pharmacol 2006;57(Suppl. 10):43–51.
[30] Lubkowska A, Szygula, Klimek A, Torii M. Do sessions of cryostimulation have influence on white blood cell count, level of IL6 and total oxidative and antioxidative status in healthy men? Eur J Appl Physiol 2010;109:67–72.
[31] Steensberg A. The role of IL-6 in exercise-induced immune changes and metabolism. Exerc Immunol Rev 2003;9: 40–7.
[32] Park HS, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNFα and IL-6. Diabetes Res Clin Pract 2005;69:29–35.
[33] Ostrowski K, Schjerling P, Pedersen BK. Physical activity and plasma interleukin-6 in humans: effect of intensity of exercise. Eur J Appl Physiol 2000;83:512–5.
[34] Nielsen AR, Pedersen BK. The biological roles of exercise-induced cytokines: IL-6, IL-8, and IL-15. Appl Physiol Nutr Metab 2007;32:833–83.
[35] Nielsen S, Pedersen BK. Skeletal muscle as an immunogenic organ. Curr Op Pharmacol 2008;8:346–51.
[36] Sessler D. Thermoregulatory defense mechanisms. Critical Care Med 2009;7:203–10.
[37] Castellani J, Young A, Duchame M, Giesbrecht GG, Glickman E, Sallis RE. ACSM position stand: prevention of cold injuries during exercise. Med Sci Sports Exercise 2006;38:2012–29.
[38] Keller C, Keller P, Marshall-Gradisnik SM, Pedersen BK. Il-6 gene expression in human adipose tissue in response to exercise: effect of carbohydrate ingestion. J Physiol 2003;550:927–31.
[39] Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissue of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. J Clin Endocrinol Metab 1998;83:847–50.
[40] Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796–808.
[41] Pedersen BK. Body mass index-independent effect of fitness and physical activity for all-cause mortality. Scand J Med Sci Sport 2007;17:196–204.
[42] Plomgaard P, Nielsen AR, Fischer CP, Mortensen OH, Broholm C, Penkowa M, Krogh-Madsen R, Erikstrup C, Lindegaard B, Petersen AM, Taudorf S, Pedersen BK. Associations between insulin resistance and TNF-alpha in plasma, skeletal muscle and adipose tissue in humans with and without type 2 diabetes. Diabetologia 2007;50:2562–71.