RESEARCH ARTICLE

Whole-body cryostimulation as an effective way of reducing exercise-induced inflammation and blood cholesterol in young men

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ABSTRACT. Inflammation may accompany obesity and a variety of diseases, or result from excessive exercise. The aim of this study was to investigate the anti-inflammatory effect of whole-body cryostimulation on the inflammatory response induced by eccentric exercise under laboratory conditions. The study also sought to establish if cold treatment changes the lipid profile and modifies energy expenditure in young people. Eighteen healthy and physically active, college-aged men volunteered to participate in the experiment. They were divided into two subgroups: CRY- submitted to whole-body cryostimulation, and CONT- a control group. Both groups performed eccentric work to induce muscle damage. Blood samples were collected before and 24 h after the exercise. Over the five days that followed, the CRY group was exposed to a series of 10 sessions in a cryogenic chamber (twice a day, for 3 min, at a temperature of -110°C). After this period of rest, both groups repeated a similar eccentric work session, following the same schedule of blood collection. The perceived pain was noted 24h after each session of eccentric workout. A 30-minute step up/down work-out induced delayed-onset muscle soreness in both groups. The five-day recovery period accompanied by exposure to cold significantly enhanced the concentration of the anti-inflammatory cytokine IL-10. It also led to a pronounced reduction in levels of the pro-inflammatory cytokine IL-1β, and reduced muscle damage. The values for IL-10 before the second bout of eccentric exercise in the CRY group were 2.0-fold higher in comparison to baseline, whereas in the CONT group, the concentration remained unchanged. Furthermore, blood concentrations of the pro-inflammatory cytokine IL-1β fell significantly in the CRY group. The main finding of this study was that a series of 10 sessions of whole body cryostimulation significantly reduced the inflammatory response induced by eccentric exercise. The lipid profile was also improved, but there was no effect on energy expenditure during the exercise.

Key words: muscle damage, cytokines, cold air exposure

Levels of physical activity have systematically decreased in developed countries in recent times, and this has become one of the main factors contributing to chronic diseases and premature death [1, 2]. Recent calculations have estimated that there are up to 5.3 million deaths/year related to low levels of physical activity [3], which can result in alteration of the blood lipid profile, induction of low grade inflammation and a decrease in the rate of energy expenditure. On the other hand, it is known that regular physical activity has an anti-inflammatory effect [4]. At the same time, provided that the homeostatic inflammatory response to exercise is sustained, vital, beneficial, adaptive changes can progress. However, this might be preceded by the interaction of free radicals, growth factors and cytokines that can accompany both injury and repair processes [5]. In particular, this kind of response can be induced by vigorous, eccentric exercise, to which the body is unaccustomed, and may lead to the chronic activation of an inflammatory response, resulting in exacerbation, rather than amelioration of the underlying damage [6]. Additionally, this type of exercise is often associated with either muscle soreness or delayed-onset muscle soreness (DOMS), usually accompanied by discomfort or pain related to muscle tenderness, stiffness or weakness [7, 8]. The most recent review published questioned the pro-healthy effect of eccentric exercise, considering it too damaging for the muscles [9].

Consequently, in the present study, this kind of exercise was chosen in order to evaluate the anti-inflammatory effect of whole-body cryostimulation. This procedure has only been used rarely or investigated in conjunction with DOMS [10, 11]. During the treatment, individuals are exposed to extremely cold, dry air (below -100°C) for

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two to four minutes [12]. The effectiveness of this method as regards reduction in inflammation however, is still considered equivocal [13, 14] and remains controversial [15, 16].

Previous investigations have shown that whole-body cryostimulation leads to an increase in the concentration of interleukin-6 (IL-6) [16, 17]. Moreover, evidence indicates that IL-6 is linked to the regulation of fat mass [18]. Cryostimulation may intensify thermogenesis to maintain a balance between heat production and loss. Moreover, data acquired by Costello indicated that both immersion in cold water and cryostimulation decreased muscle and rectal temperatures in similar ways [19]. Additionally, it was revealed that a series of 20 cryostimulation sessions significantly altered the lipid profile in healthy subjects, reducing the levels of total cholesterol, low-density cholesterol and triglycerides, yet increasing the levels of high-density cholesterol [20]. These results suggest that whole-body cryostimulation may influence energy expenditure, which could reinforce the beneficial effect of exercise. We hypothesised that whole body cryostimulation might reduce the inflammation induced by eccentric exercise, improve the lipid profile and increase energy expenditure.

VOLUNTEERS AND METHODS

Subjects

Eighteen healthy and physically active, college-aged men volunteered to participate in the experiment. The subjects were fully informed of the risks and stresses associated with the study and gave their written consent to participate. The Bioethical Committee of the Regional Medical Society in Gdansk NKEBN/245/2009 issued its formal approval of the study, according to the Helsinki Declaration. One week prior to commencing the experiment, body composition and aerobic capacity were determined for each participant. According to the maximal oxygen capacity values, the participants were randomly paired and assigned to either the cryostimulated group (CRY; n = 9, 21.7 ± 0.9 years-old) or the control group (CONT; n = 9, 22.0 ± 2.0 years-old). None of the participants in the CRY group had previous experience of whole-body cryostimulation.

The main part of the experiment consisted of a 30-minute session of step up/down exercise, aimed at inducing DOMS. Over the next five days, the CRY group was subjected daily to whole-body cryostimulation (twice a day), while the CONT group rested without any recovery–supporting treatment. No physical activity was undertaken by the two groups during these five days. Subsequently, all participants repeated the step up/down exercise with the same intensity as in the first session. The investigation schedule is presented in *figure 1*.

Anthropometric measurements

Body mass (BM) and composition were estimated using a bioelectrical impedance (TBF-300 Body Fat Monitor/Scale Analyzer, Tanita, Japan) floor scale with an accuracy of 0.1 kg. The measurements were taken one hour before breakfast. Participants had voided their bladders and bowels prior to the assessment. During the measurements, subjects wore only briefs and remained barefoot. The device was calibrated prior to each measurement session. Data accuracy was 98% [21].

Aerobic power measurement

The examination aimed to establish the level of physical capacity, expressed as the maximal oxygen consumption (VO_2 max) and the maximal aerobic power. Breath-by-breath pulmonary gas exchange was measured (MetaMax 3B, Cortex Biophysik, Germany) throughout the exercise. Participants performed a graded test on an electromagnetically-braked,cycle ergometer (ER 900 Jaeger, Germany/Viasys Health Care). Participants were alloweda five-min warm-up at an intensity of 1.5 W/kg, with a pedalling cadence of 60 rpm. Immediately following the warm-up, the VO_2 max test was begun. Each participant cycled with an increasing workload until they reached the point of volitional exhaustion. The resistance was increased at a rate of 25 W/min. The O_2 and CO_2 analysers were calibrated prior to each test using standard gases of known concentrations in accordance with the manufacturer's guidelines [21].

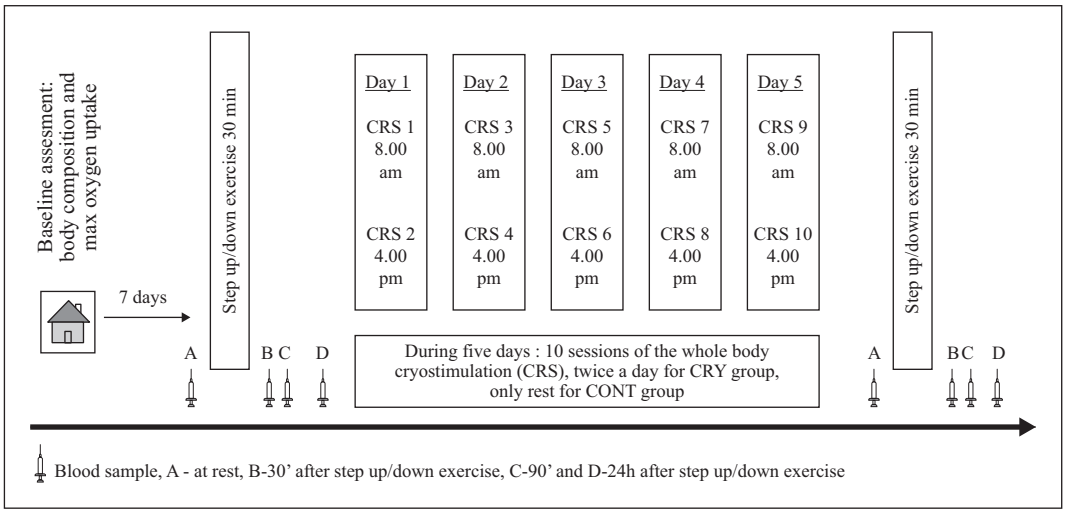

Figure 1

Schematic presentation of the experimental design.

Muscle damage

Both groups performed a 30-minute step up/down exercise (involving mainly eccentric work of the muscle fibres) at 60% of maximal aerobic power. Based on the individual values of maximal power, the up/down step frequency was calculated to ensure that this intensity level was maintained throughout the test [22, 23]. The step was 51cm high and the average step frequency was equal to 20 steps per minute. Breath-by-breath pulmonary gas exchange was measured (MetaMax 3B, Cortex Biophysik, Germany) throughout the exercise. Indirect calorimetry was used to assess non-protein substrate oxidation [24]. Additionally, to record the physiological energy cost of the activity performed, the respiratory exchange ratio (RER), the metabolic equivalent (MET) and the heart rate (HR) were measured continuously.

Whole-body cryostimulation

The CRY group was subjected to a series of exposure to cold 24 h after performing the step up/down exercise. Having analysed the effectiveness of different whole-body cryostimulation protocols, a procedure involving 10 sessions (5× twice a day at 9:30am and 15:00pm) was instigated [17]. The treatment was administered in a cryogenic chamber at the Pomeranian Rheumatologic Centre in Sopot, Poland, and was carried out by highly qualified medical staff. Each session lasted three minutes at a temperature of -110°C. Each entry into the cryo-chamber was preceded by a 20-30s adaptation in the vestibule at a temperature of -60°C. Subjects wore shorts, socks, gloves and a hat that covered their ears. They did not participate in any treatment other than the whole-body cryostimulation to avoid obscuring the interpretation of the cryogenic effect. Each cryo-session was preceded by a light breakfast, between 7:00 and 7:30am, according to the instructions given to the participants.

Blood analysis

Blood samples were collected to determine the haematological parameters and to measure the pro- and anti-inflammatory cytokine levels and CK activity. These were taken at baseline, 30 min, 90 min, 24 h, 48 h and 72 h after each session of step up/down exercise (before and after cryostimulation). However, results recorded 48 h and 72 h following both work-outs are not presented because of their lack of statistical significance. *Figure 1* presents an overview of the points of blood collection for determining the haematological parameters (point A), for the cytokine concentrations (points A and D) and for the CK activity (points A, B, C, D). The samples were always collected between 8:00 and 8:30am from the antecubital vein (*v. mediane cubiti*). After collection, they were immediately stored at a temperature of 4°C. Within 10min, they were centrifuged at 2500 g and 4°C for 10 min. Aliquots of plasma were stored at -70°C.

The haematological measurements were performed using conventional methods with a Coulter® LH 750 Hematology Analyzer (Beckman-Coulter, USA).

Plasma CK activity was used as a marker of muscle damage and was evaluated using an Emapol kit (Poland). The CK detection limit of the applied kit was 6 U/L. The intra-assay CV for the CK kit was 1.85%. The serum concentrations of total cholesterol (TCH), HDL cholesterol and triglycerides (TG) were determined with commercial kits using enzymatic methods (Alpha Diagnostics, Poland). LDL cholesterol was calculated using the Friedewald formula.

Plasma interleukin (IL-6, IL-10, IL-1β) concentrations were determined by enzyme immunoassay methods using commercial kits (R&D Systems, USA, catalogue no. HS600B, HS100C, HSLB00C, respectively). The detection limits for IL-6, IL-10 and IL-1β were 0.039, 0.500 and 0.023 pg/mL, respectively. The average intra-assay CV was <8.0% for all cytokines.

Perceived pain

Both groups assessed the perceived pain 24h after each of the two sessions of step up/down exercise (before and after the cryostimulation), using a visual scale (VAS). This is a 10-cm-long labelled line, graded from left to right, ranging from "no soreness" to "extremely sore". Its accuracy is estimated to the nearest 0.1 [25, 26]. The pain was assessed for the quadriceps muscle and legs muscle in general. Prior to the evaluation, participants were familiarised with the method in accordance with the standard guidelines. They were instructed to mark the level of pain they experienced. Both parts of the evaluation (quadriceps and overall) were repeated twice a day, always using a fresh scale to avoid biased interpretations. Next, the data gathered were averaged. The VAS measurement accuracy was 99%.

Statistical analysis

The statistical analysis was performed using Statistica 8.0 for Windows. A 2 (group) × 2 (time) repeated measures analysis of variance (ANOVA) was used to determine the significance of the differences between the groups, as well as before and after whole-body cryostimulation. Data normality was tested using the Shapiro-Wilks W-test. Statistical significance was set at $p < 0.05$ for the whole analysis. Additionally, to elaborate on the significance of the difference between the groups before and after whole-body cryostimulation, the multiple comparison method (*post hoc* – HSD Tukey's) was used. Furthermore, to assess the influence of whole-body cryostimulation, the effect size was calculated (partial eta^2) by ANOVA, ranging between 0 and 1. For some parameters, associations between measured parameters were analysed using Pearson's linear regression (coefficient, r).

RESULTS

All participants completed the study with no adverse effects being reported. The basic anthropometric and physiological characteristics of the subjects are summarised in *Table 1*. Repeated measurements indicated that no differences in the anthropological parameters before and after the cryostimulation intervention were noted between the groups. Moreover, no differences in the cytokine concentrations or the CK activity were recorded between the groups at baseline (*figures* 2A, 3A, 4A).

Table 1
Anthropometric and physiological characteristics of participants.

Variable	CRY group						CONT group					
	I			II			I			II		
Height [cm]	180.7	±	5.9	nd			183.8	±	6.5	nd		
Weight [kg]	78.7	±	7.3	78.6	±	7.6	77.4	±	9.2	77.3	±	9.1
FFM [kg]	66.4	±	4.8	66.1	±	5	66.5	±	6.2	66.5	±	6.2
TBW [kg]	48.6	±	3.5	48.4	±	3.6	48.7	±	4.6	48.7	±	4.5
Fat [kg]	12.3	±	3.4	12.5	±	3.6	10.9	±	3.3	10.8	±	3.4
Fat %	15.4	±	3.4	15.6	±	3.6	13.8	±	2.7	13.8	±	2.7
BMI [kg/m^2]	24.1	±	2.7	24	±	2	22.9	±	1.7	22.9	±	1.7
$VO_{2\ max}$ [mL/kg.min]	50.2	±	4		nd		52	±	8	nd		
Power max [W/kg]	3.6	±	0.6		nd		3.8	±	0.3	nd		
HR [b/min] max	187	±	6		nd		190	±	7	nd		

Values are means ± SD. CRY: cryostimulation group; CONT: without cryostimulation group; I: before the first eccentric work; II: before the second eccentric work; no statistical differences for group or time before and after sessions of cryostimulation; nd: not determined; Fat: fat mass; FFM: free fat mass; TBW: total body water; BMI: body mass index; VO2: maximal oxygen consumption; HR: heart rate.

Inflammatory response and muscle damage after the first step up/down exercise

The 30-minute step up/down exercise induced DOMS in both groups. Creatine kinase activity was increased in all participants. The analysis of repeated measurements indicated that in both groups, values recorded 24 h after the exercise confirmed muscle damage (*figure 2A*). Furthermore, the presence of DOMS was demonstrated by the perceived pain evaluation at this point. All participants experienced the same level of pain. In the CRY group, VAS was 6.4 ± 1.1cm, whereas in the CONT group, it was 6.2 ± 1.3 cm. Additionally, the step up/down exercise triggered an immunological response in all subjects. Compared to baseline, the concentration of IL-6 in the blood samples taken 24h afterwards increased 2.8-fold and 3.9-fold in the CRY and CONT groups, respectively (*figure 3A*). At the same time, increased concentrations of the pro-inflammatory cytokine (IL-1β) were recorded. Values increased 3.9-fold and 3.7-fold in the CRY and CONT group, subsequently (*figure 4A*). On the other hand, the anti-inflammatory cytokine IL-10 increased 3.8-fold in the CRY group and 3.2-fold in the CONT group (*figure 4C*).

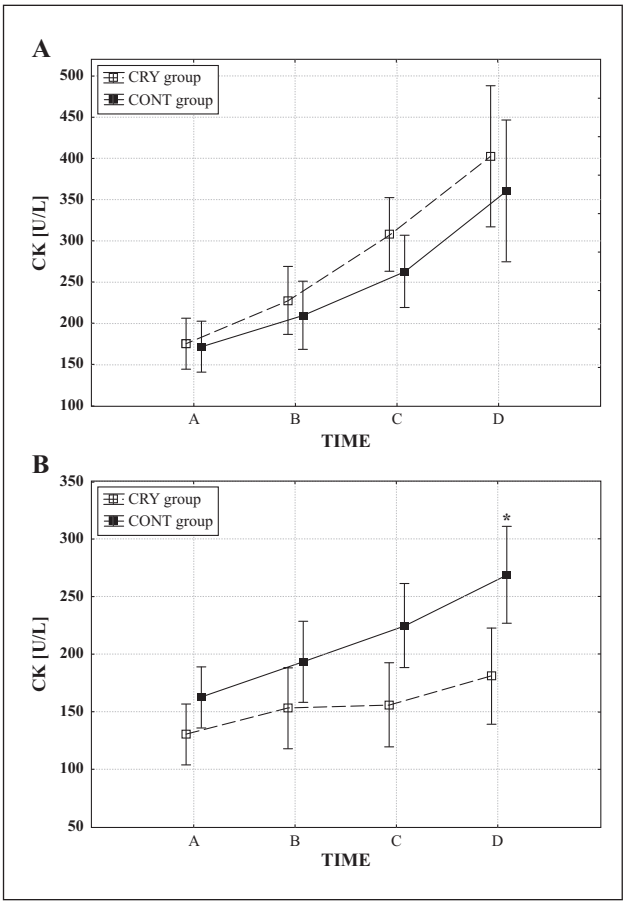

Figure 2
Plasma CK activity after first **(A)** and second **(B)** step up/down exercise, * differences between groups, ¶ differences within group in comparison to baseline; data significant different at 0.05.

Changes in cytokine concentrations in response to 10 sessions of whole-body cryostimulation and five days rest

The five days of recovery including, whole-body cryostimulation in the CRY group, effectively altered the concentration of the anti-inflammatory cytokine IL-10. Values recorded after this period, were 2.0-fold higher in comparison to baseline, whereas in the CONT group, the concentration of IL-10 remained unchanged (*figure 4D*). At the same time, blood concentrations of the pro-inflammatory cytokine IL-1β dropped significantly in both groups. It is worth noting that after the five-day rest period IL-1β values were significantly different between the two groups ($p = 0.0001$). The concentration in the CRY group fell from 2.2 pg/mL to 0.4 pg/mL, whereas in the CONT group it dropped only by half from 2.4 pg/mL to 1.2 pg/mL (*figure 4B*). In contrast to previous observations [17, 27], the whole-body cryostimulation protocol did not induce an increase in the levels of IL-6 (*figure 3B*). Both groups had slightly elevated values before the second step up/down exercise session; however, the inter-group differences were not statistically significant in comparison to baseline.

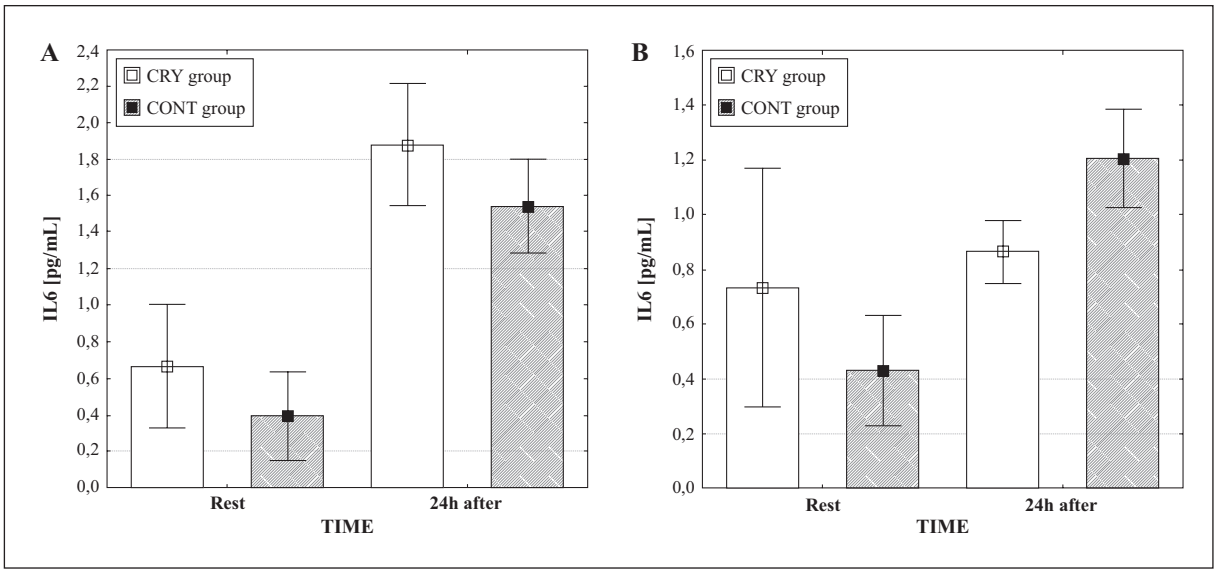

Figure 3
The concentration of IL-6 interleukin at baseline and 24h after two sessions of step up/down exercise : **(A)** first, **(B)** second.

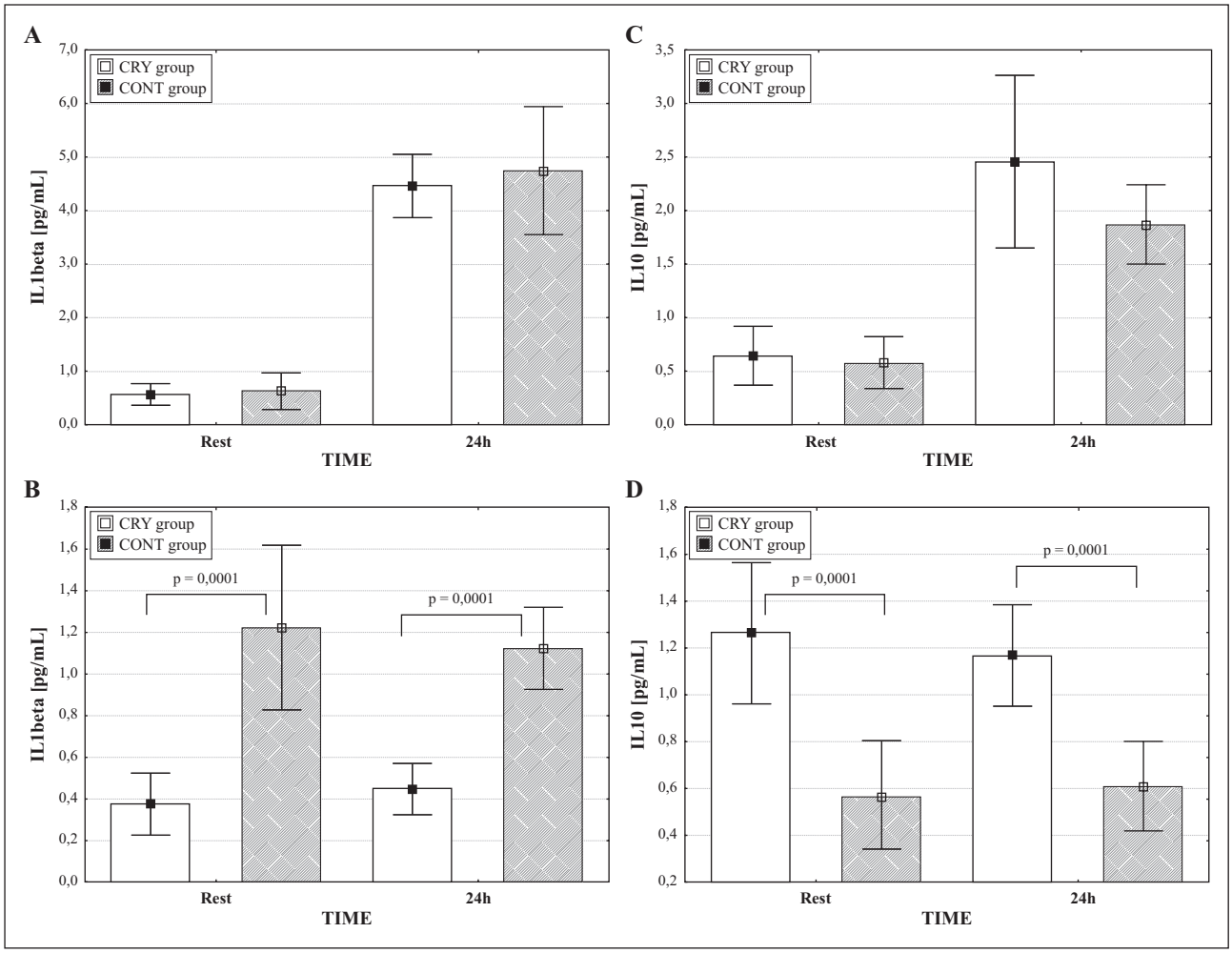

Figure 4
The pro-inflammatory cytokine IL-1β, at baseline and 24h after two sessions of step up/down exercise: **(A)** first, **(B)** second. The anti-inflammatory response of IL-10 to exercise: **(C)** first, **(D)** second.

Changes in haematological parameters

The series of whole-body cryostimulation sessions during the five-day rest period had an effect on the haematological parameters. Significant changes were observed in the percentage of basophils, whose numbers increased in the CRY group following the procedure. Interestingly, the calculated effect size for this measurement indicated that cryostimulation accounted for a change of 45%. Moreover, the treatment affected the cholesterol level. Concentrations of

Table 2
Metabolic rate during eccentric exercise before and after 10 sessions of whole body cryostimulation.

		CRY group						CONT group					
Variable	**Phase**	**I**			**II**			**I**			**II**		
EE [kcal]	1	432.2	±	65.5	428.9	±	47	423.7	±	50	426.3	±	59.5
EE [kcal/kg]	1	5.5	±	1	5.5	±	0.7	5.5	±	0.6	5.5	±	0.6
CHO [g]	1	68.7	±	25	55.7	±	9	63.7	±	17.4	58.2	±	19
CHO [g/kg]	1	0.9	±	0.3	0.7	±	0.1	0.8	±	0.2	0.7	±	0.2
FAT [g]	1	8.6	±	6.2	14.1	±	6.3	10	±	7.8	13	±	7.6
FAT [g/kg]	1	0.1	±	0	0.2	±	0	0.1	±	0	0.2	±	0.1
MET	1	10.4	±	1.7	10.4	±	1.2	10.6	±	1.3	10.8	±	1.2
HR [b/min]	1	153	±	18	147	±	14	154	±	14	150	±	18
RER	1	0.91	±	0	0.87	±	0	0.91	±	0	0.89	±	0
EE [kcal]	2	461	±	31.7	455.7	±	45.3	470.9	±	61	480.2	±	70.4
EE [kcal/kg]	2	5.9	±	0.7	5.8	±	0.6	6.1	±	0.7	6.3	±	1.3
CHO [g]	2	71.1	±	15.5	53.2	±	11.2	66.8	±	26.7	63.7	±	28.9
CHO [g/kg]	2	0.9	±	0.2	0.6	±	0.1	0.8	±	0.3	0.8	±	0.4
FAT [g]	2	10.2	±	6.5	17.5	±	6	13	±	9.6	15.2	±	10.3
FAT [g/kg]	2	0.1	±	0	0.2	±	0	0.2	±	0.1	0.2	±	0.1
MET	2	11.2	±	1	11	±	0.8	11.8	±	1.4	12.3	±	2.4
HR [b/min]	2	176	±	13	169	±	14	174	±	8	175	±	12
RER	2	0.9	±	0	0.86	±	0	0.89	±	0	0.89	±	0

Values are means ± SD. CRY: cryostimulation group; CONT: without cryostimulation group; I: first eccentric work; II: second eccentric work; EE: energy expenditure; CHO: carbohydrate; FAT: fat; MET: metabolic equivalent; HR: heart rate; RER: respiratory exchange ratio; 30 min exercise phase; 1: first 10 minutes during achieving steady state; 2: last 10 minutes of eccentric exercise; no statistically differences for group and time before and after cryostimulation,

total cholesterol and low-density lipoprotein dropped considerably, with effect sizes of 43% and 52%, respectively (*Table 3*). Additionally, LDL/HDL and total cholesterol/HDL ratios were calculated. The series of whole body cryostimulation only modified the LDL/HDL ratio in the CRY group, causing it drop, whereas in the CONT group the ratio increased. These changes were statistical significant. Also the increased, post-cryostimulation triglyceride levels in the CRY group correlated positively with the average RER during the second session of eccentric exercise ($r = 0.82$, $p = 0.05$).

Effects of 10 sessions of whole-body cryostimulation on exercise-induced muscle damage and the inflammatory response.

The second session of exercise after the exposure to cold induced an increase in blood CK activity. Nonetheless, 24h after the second session of the step up/down exercise, the values were lower in the CRY group than in the CONT group ($p = 0.001$). Moreover, in the CONT group these increased values were statistically significant compared to the baseline (*figure 2B*). Perceived pain was 2.9 ± 1.7 cm in the CRY group, whereas in the CONT group, it was higher, 4.2 ± 1.0 cm. The differences were statistically significant ($p = 0.05$).

The exposure to extremely low temperatures affected the immunological response to the second session of the step up/down exercise. As a consequence, the increased concentration of the anti-inflammatory cytokine IL-10 was maintained 24 h after exercise in the CRY group, whereas in the CONT group, it did not change in comparison to the values before the second step up/down exercise (*figure 4D*). Moreover, the second work session did not trigger significant changes in the concentration of pro-inflammatory IL-1β in comparison to the values recorded after five days of rest. Nevertheless, the CRY group was characterised by significantly lower concentrations of IL-1β 24h after exercise (*figure 4B*). Interestingly, the second step up/down exercise caused smaller increases in IL-6 in both groups (*figure 3B*).

Effect of whole-body cryostimulation on the physiological cost of the second step up/down exercise

Physiological parameters recorded during the 30-minute step up/down exercise indicated that there were no significant differences in energy expenditure (RER, EE) or physiological response (MET and HR) between the exercise sessions (*table 2*). Interestingly, during the second work-out in the CRY group, the absolute amount of oxidised fat increased in the first and second phase of the exercise (50% and 71% respectively). The rise observed in the CONT group was much lower. The increased fat oxidation rate was accompanied by a decrease in carbohydrate oxidation. However, these differences were not statistically significant (*table 2*).

DISCUSSION

The main findings of this study were that the 10 sessions of whole body cryostimulation significantly reduced the inflammatory response induced by eccentric exercise; improved the lipid profile, but had no effect on the exercise energy expenditure. It is known that inflammation may accompany obesity [28] and various diseases [29, 30], or might result from excessive exercise [31-33].

The main goal of the study was to evaluate the influence of whole body cryostimulation on the inflammation induced by step/down exercise under laboratory conditions. This kind of exercise has been previously reported to induce muscle damage and a forced inflammatory response [23]. Thus, we had assumed that this could be a good experimental model for investigation of the anti-inflammatory effect of whole body cryostimulation.

The data obtained revealed that the first step/down exercise session induced inflammation, manifesting as an increase in the concentration of the pro-inflammatory cytokine IL-1β. Several reports have indicated that plasma IL-1β does not change in response to exercise [34-36], while others observed that any slight increase was short-lived [37]. On the other hand, a 30-minute session of eccentric cycling caused an increase in the level of IL-1β in muscle biopsies [38]. Therefore, we concluded that the noted rise of IL-1β resulted from the muscle damage rather than the exercise *per se*. Also, given the greater decrease in IL-1β in the CRY group, the rest period supported by the cryostimulation must have enhanced subjects' recovery rate. What is more, the series of whole body cryostimulation had stimulated some adaptive changes, which had enhanced muscle resistance to damage induced by the second step-up/down exercise session. Interestingly, we noted that after the second exercise session, CK activity declined in both groups, with the reduction seen in the CRY group being more pronounced. Nosaka *et al.* reported that muscle damage due to eccentric exercise triggers protein synthesis, which enhances muscle protection against future, exercise-induced muscle damage [39]. Additionally, the perceived pain was significantly lower in the CRY group. These findings are in agreement with previously published papers that had documented the analgesic and anti-inflammatory effect of whole-body cryostimulation [15, 40, 41]. Our data revealed that cryostimulation might induce an effect similar to cold water immersion, which reduces cell necrosis and neutrophil migration, limiting secondary damage [42].

The whole-body cryostimulation protocol applied during the recovery period significantly improved the anti-inflammatory response expressed as an increase in IL-10 levels. The anti-inflammatory mechanism of IL-10 has been discussed previously in studies on cell culture, which reported a decreased expression of a wide spectrum of pro-inflammatory cytokine genes, brought about by IL-10 [43, 44]. In the present study, the concentration of IL-10 had risen after the first session of the step-up/down exercise in both groups. Interestingly, in the CRY group, it remained elevated after the five-day rest period as well as after the second session of the step-up/down exercise, whereas in the CONT group it reached baseline level after the second bout of exercise. Therefore, the decline observed in the level of the pro-inflammatory IL-1β in the CRY group might have been caused by the increased level of IL-10. A similar effect was noted by Banfi and co-workers in group of rugby players who underwent regular, moderate training in conjunction with five cryostimulation sessions [40].

The wide range of effects that whole-body cryostimulation may trigger results from an equally wide variety of methodological practices applied during exposure. The variety of factors include engaging different patients [41, 45], as well as applying a different frequency [41, 46, 47] and/or number of exposures [16, 46]. Furthermore, data published on the morphological parameters recorded after cryotherapy, are inconsistent. In our investigation, no significant changes in the haematological parameters were observed, except for the percentage of basophils in the CRY group. A similar observation was noted in a previous report using a similar procedure of whole-body cryostimulation [17].

The second aim of this study was to see if twhole-body cryostimulation could change the lipid profile in young people. An increasing number of studies have shown that unfavorable changes in the lipid profile can be seen not only in obese [48] or older subjects [49], but also in young, physically inactive people [48-50]. In our study, the procedure caused decreases in the levels of total cholesterol and low-density lipoprotein. These results are in agreement with previous observations by Lubkowska and co-workers [20]. Moreover, the cryostimulation protocol applied significantly decreased the LDL/HDL ratio. Some papers have reported that ratios between the fraction of cholesterol and total cholesterol might be considered as reliable indicators, not only of coronary disease [51], but also of insulin sensitivity [52, 53]. However, further research, involving many muscle biopsies, would be necessary to draw such broad-based conclusions.

Our data did not corroborate our primary hypothesis that whole-body cryostimulation would cause the concentration of IL-6 to increase. The elevated values of IL-6 observed 24 h after the first bout of exercise in all subjects were induced by eccentric work, which is in agreement with the report published by Pedersen and Febbraio [54]. Although IL-6 has been previously demonstrated to have increased in response to cold, [16, 17, 47], in our experiment, the procedure did not cause a similar effect. It is the differences in body fat content that might have influenced the response to cryostimulation. At the same time, analysis of the absolute amount of fat oxidised during each phase of exercise suggests that fat metabolism was increased in the CRY group. Additionally, the physiological parameters (HR and MET) exhibited by the CRY group were lower compared to the CONT group, yet the differences were not statistically significant. It is possible that the procedure did not trigger an effect significant enough to alter the energy expenditure during the second bout of exercise. A recently published paper by Costello [19] suggested that whole body cryostimulation decreased both muscle and skin temperatures, causing an effect similar to that induced by cold water immersion. However, these changes although significant, only persisted for the 60 minutes that followed immersion. This might explain the lack of effect of whole body cryostimulation on energy expenditure during the exercise session performed one day after the cold treatment in our study.

Undoubtedly, our experiment would have benefited from a functional assessment; however, the purpose of the study

Table 3
Hematological parameters before and after 10 sessions of whole body cryostimulation.

Variable	CRY group I	CRY group II	CONT group I	CONT group II	Differences	*P* Value	Effect size η_p^2
White blood cells [10^3 μL]	5.4 ± 1.0	5.0 ± 1.6	5.7 ± 1.0	5.7 ± 1.1	ns		
Lymphoctes [%]	34.0 ± 8.6	31.2 ± 7.8	38.5 ± 4.8	38.6 ± 4.8	ns		
Basophils [%]	0.4 ± 0.2 [a]	0.9 ± 0.4 [b]	0.5 ± 03	0.5 ± 0.2[b]	time time × group	0.007 0.002	0.38 0.45
Neutrophils [%]	53.0 ± 9.9	55.9 ± 9.0	48.7 ± 6.4	49.1 ± 6.6	ns		
Monocytes [%]	10.2 ± 2.3	9.3 ± 2.0	9.7 ± 2.0	9.6 ± 2.1	ns		
Eosinocytes [%]	2.3 ± 1.3	2.7 ± 1.3	2.5 ± 1.0	2.2 ± 1.0	ns		
Thrombocytes [10^3 μL]	13.2 ± 0.4	12.9 ± 0.4	13.5 ± 2.4	13.5 ± 2.4	ns		
Red blood cells [10^3 μL]	5.0 ± 0.3	4.9 ± 0.7	4.9 ± 0.3	4.9 ± 0.3	ns		
Hematocrit [%]	43.7 ± 2.1	42.6 ± 1.7	44.1 ± 2.0	44.0 ± 2.0	ns		
Hemoglobin [g/dL]	15.0 ± 1.0	14.4 ± 0.8	15.3 ± 0.7	15.2 ± 0.7	ns		
Total Cholesterol [mg/dL]	163.9 ± 18.8	147.1 ± 16.1	169.1 ± 26.7	173.6 ± 31.7	time × group	0.003	0.43
HDL [mg/dL]	59.3 ± 12.9	58.9 ± 14.1	59.4 ± 13.5	56.2 ± 13.2	time	0.005	0.38
LDL [mg/dL]	88.2 ± 14.7	74.7 ± 13.9	89.1 ± 18.6	96.7 ± 22.5	time × group	0.0007	0.52
Triglycerides [mg/dL]	81.8 ± 39.2	87.9 ± 44.7	102.9 ± 31.9	104.1 ± 40.3	ns		
LDL/HDL ratio	1.6 ± 0.6	1.5 ± 0.5	1.5 ± 0.3	1.8 ± 0.5	time × group	0.0007	0.37
T-CH/HDL ratio	2.8 ± 0.7	2.8 ± 0.5	2.9 ± 0.4	3.1 ± 0.6	ns		

Values are means ± SD. HDL: high density lipoprotein; LDL: low density lipoprotein; T-Ch: total cholesterol; I: at the baseline, II after 10 sessions of cryostimulation for the CRY group and without of exposure for the CONT group; ns: no statistical differences for group and time before and after cryostimulation; : effect size expressed as partial *eta*-2; a: differences in the group CRY before and after exposures; b: after cryostimulation differences between groups.

was to evaluate indicators that directly correspond to muscle damage, inflammation and lipid profile. Muscle strength or muscle endurance could be affected not only by physical, but also by psychological factors; therefore, the study focused on the biochemical assessment only.

In summary, we can conclude that whole-body cryostimulation reduced both inflammation and muscle damage induced by the step-up/down exercise. Our data strongly suggest that this effect was mediated by IL-10. This is the first time that whole body cryostimulation has been shown to have sustained an increased level of IL-10 after exercise with an eccentric component. Moreover, this procedure could be used to modify the lipid profile. That whole body cryostimulation reduces inflammation and blood cholesterol, as well as having an analgesic effect, might justify further medical applications.

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