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The effect of whole-body cryostimulation on the activity of lysosomal enzymes in kayaker women after intense exercise

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ABSTRACT

In this study higher activity of certain lysosomal enzymes with concomitant lower α_1-antitryspin activity was revealed in serum of kayaker women after intense exercise without any external stimuli as compared with the exercise preceded by extreme cold application. Whole-body cryostimulation may have hormetic, beneficial impact on reduction of muscle damage.

1. Introduction

In professional sport the procedures of the optimization and personalization of training conditions are very common. All of those modalities are aimed to protect the organism of sportsman against any stressor agents that may occur during the exercise. In this context a modern training may lead to the deficiency of natural factors that stimulate the organism, such as thermal stimuli. Therefore the sports medicine searches for the methods to prevent the negative effects of natural stimuli deficiency. Such a procedure seems to be the whole-body cryostimulation (WBC), which is defined as a short-term exposure of whole organism to extreme cold in a special temperature-controlled chamber (Banfi et al., 2009). WBC per se is known to be a stressor agent for organism and among others it induces oxidative stress (Woźniak et al., 2007). Thus, according to the hormetic theory, brief application of whole-body cryostimulation may make the organism adapted to different stressor agents, like intense exercise (Mila-Kierzenkowska et al., 2009). Such adaptation leads to an improved ability to resist the negative effects of training including the damage of muscle fibres.

An intense exercise is widely known to cause the imbalance of organism homeostasis. Among many factors that may affect the cellular homeostasis, the alterations in the lysosomes seem to be of great importance (Schott and Terjung, 1979). Lysosomes are the organelles that contain lots of hydrolases, which are the enzymes responsible for breaking down complex chemicals within a cell, which have expended their useful life therefore they play a crucial role in cellular repair from the injury (Bakońska-Pacoń et al., 2005). The acute exercise may be a reason of an increased lability of lysosomal membranes and the release of lysosomal hydrolases into the cytoplasm, extracellular matrix and then into the blood system (Woźniak et al., 2007). The best known protease occurring within the lysosomes is cathepsin D (CTSD), which is the enzyme that catalyze the reaction of proteolysis of extra- and intracellular proteins (Tsukuba et al., 2000). It also participates in apoptosis induced by oxidative stress (Kagedal et al., 2001). The other lysosomal enzymes are also acid phosphatase (AcP), which is a biochemical marker of lysosomes damage (Gregoraszczuk and Sadowska, 1997) and arylsulphatase (ASA) used for the evaluation of the level of muscle fatigue (Bakońska-Pacoń et al., 2005).

The physical exercise of long duration and/or high intensity is also reported to induce the acute phase response. This phenomenon is a generalized systemic response that protects the body and helps to restore the homeostasis and it includes a wide range of pro- and anti-inflammatory proteins (Semple et al., 2006). One of the anti-inflammatory compounds of the acute phase response is α_1-antitrypsin—AAT (Semple et al., 2006). This protein is a serine proteinase inhibitor (Zhang et al., 1993) that has the ability to inhibit certain proteinases, including some of lysosomal hydrolases

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(Janciauskiene and Lindgren, 1999). The elements of the organism response to exercise are also well-known and commonly used in sport markers like creatine kinase (CK) and cortisol. The serum CK level may be increased as a consequence of muscle damage after the intense exercise, both by metabolic and mechanic causes (Brancaccio et al., 2007).

The whole-body cryostimulation is already acknowledged method of improving recovery after intense training, but specific studies on it are still lacking. The purpose of this study was to evaluate the effect of the whole-body cryostimulation on the activity of some lysosomal enzymes and creatine kinase as well as the cortisol level in blood serum of kayaker women who performed intense exercise.

2. Material and methods

The study was conducted on a group of 9 kayaker women of the Polish Olympic Team. The characteristic of investigated group is shown in Table 1. The sportswomen performed two typical ten-day training cycles: one without application of whole-body cryostimulation and the other with twice a day exposure to cryogenic temperature (the training protocol is shown in Table 2). The training cycle without WBC took place four months before the start of an experiment with application of extremely low temperatures. Blood samples were obtained from subjects before the start of the study (control) as well as after the 6th and the 10th day of both training cycles. The blood samples were taken from the cubital vein in the morning, after overnight fasting and were put into the sterile tube.

The investigated group of kayaker woman underwent twenty cryotherapy sessions over a period of 10 days. Each day, the first session was performed in the morning, before the start of exercise, while the second one just before the next training session. The cryo-chamber was cooled with liquid nitrogen, and the temperature was monitored by computer. Every sportswoman entering the cryo-chamber was dressed in two piece swimsuit, socks, gloves, a headband and wooden clogs and was wearing a surgical mask over the nose and mouth in order to avoid the frostbites. Before the entry to cryochamber the subjects were informed of the necessity of taking slow, shallow breaths. Before each 3 min long entry to proper cryo-chamber with the temperature ranging from -120 to $-140\ ^{\circ}C$, the kayaker women underwent a few-second adaptation period at temperature of $-60\ ^{\circ}C$, being all time in contact with the person supporting the treatment.

The research has gained the agreement of the Bioethics Committee at Collegium Medicum in Bydgoszcz of the Nicolaus Copernicus University in Toruń and all sportswomen subjected to the study gave their written informed consent.

In blood serum the activity of acid phosphatase (AcP), arylsulphatase (ASA), cathepsin D (CTSD), α_1-antitrypsin (AAT) and creatine kinase (CK) as well as cortisol level were evaluated. AcP activity was determined by Bessey's method (Krawczyński, 1972). The activity measure was the quantity of p-nitrophenol generated during enzymatic hydrolysis of 4-nitrophenylophosphate disodium salt used as a substrate. The activity of ASA was assayed according to Roy's method modified by Błeszyński (Błeszyński and Działoszyński, 1965). The substrate was 4-nitrocatechol sulphate and the quantity of 4-nitrocatechol released during enzymatic hydrolysis of a substrate was measured. CTSD activity was determined using the Anson method based on the measurement of tyrosine quantity released during hydrolysis of haemoglobin by CTSD (Colowick and Kaplan, 1955). The activity of AcP, ASA and CTSD was expressed in nanokatal (nkat). The activity of α_1-antitrypsin (AAT) was determined accordingly to Eriksson (Eriksson, 1965; Szmidt et al., 1991). The procedure is based on the evaluation of amount of trypsin inhibited by AAT present in 1 ml of blood serum and the activity was expressed in mg trypsin/ml of serum. Creatine kinase activity was measured by Oliver method (Oliver, 1955) with the modification of Szasz (Szasz et al., 1976), with application of ready-to-use diagnostic kits from Alpha Diagnostic sp. z o.o., Warsaw. The activity of CK was expressed in IU/l. The level of cortisol was determined by method based on chemiluminescence with the use of Immulite machine and expressed as μg/dl (Tello and Hernandez, 2000).

During the training events the intensity of training was measured by blood lactate level (Table 2). The concentration of lactate was evaluated with use of the portable lactate analyzer—Lactate Scout (EKF—diagnostic GmbH, Magdeburg, Germany).

All results were statistically analysed by means of one-way ANOVA test and the changes on the level of significance $p < 0.05$ were considered as statistically significant.

Table 1
The basic characteristics of kayaker women.

Number of subjects	9
Age (years)	23.9 ± 3.2
Height (cm)	167.0 ± 4.5
Weight (kg)	63.1 ± 4.1
Training experience (years)	11.7 ± 2.4
VO_{2max} (ml min^{-1} kg^{-1})	56.4 ± 2.6

3. Results

The training cycle without cryostimulation induced the increase in activity of acid phosphatase – about 80% ($p < 0.01$) and arylsulphatase – about 83% ($p < 0.01$) after first six days of training (Table 3). After 10th day of training the activity of those enzymes slightly decreased, but still it was higher than the control value (difference statistically insignificant). In the course of training preceded with twice a day cryostimulation there were no statistically significant changes in both AcP and ASA activity in blood serum of kayaker women as compared with the value before the start of the study and those activities were lower than in the course of training without cryostimulation. After the sixth day of training without cryostimulation the AcP activity was about 67% ($p < 0.01$) and ASA activity about 77% ($p < 0.01$) higher than at the same point of training cycle but supported with whole-body cryostimulation. After the tenth day there were no statistically significant differences between different training cycles.

After the tenth day of training without cryostimulation in blood serum of investigated subjects more than two-fold increase ($p < 0.01$) of cathepsin D activity was revealed with concomitant decrease of protease inhibitor (α_1-antitrypsin) activity (Table 3). The AAT activity after sixth day of training was almost three-fold lower ($p < 0.01$) and after tenth day it deceased even more and was about eight-fold lower as compared with the control activity (before the start of the study). During the training with cryostimulation more then two-fold increase of CTSD was observed in blood serum of investigated kayaker women after the tenth day as compared with the control value ($p < 0.001$). This activity was also higher versus sixth day of training both without ($p < 0.01$) and with exposure to the cryogenic temperatures ($p < 0.001$). AAT activity during the training with application of cryochamber sessions did not differ from the value before the start of the training and it was three-fold higher ($p < 0.01$) after the sixth day and more than seven-fold higher ($p < 0.05$) after the tenth day as compared with related activity after the training without cryostimulation.

In the whole course of investigated training cycles statistically significant increase in creatine kinase activity was revealed as compared with the value before the start of the experiment

Table 2
Training protocol of kayaker women during two ten-day training cycles: without whole-body cryostimulation and preceded with twice a day cryostimulation.

	Training cycle without cryostimulation		Training cycle with twice a day cryostimulation	
Day of week	**Type of training**	**Time and intensity**	**Type of training**	**Time and intensity**
Mon.	General training	120 min II band	General training	120 min I band
	Strength training	90 min I/II band	Strength training	90 min I band
Tues.	General training+endurance training	120 min II/III band	Training on ergometer	120 min I band
	General training+endurance training	120 min II/III band	General training	120 min I/II band
Wed.	Maximum strength training	90 min I/II band	Sports, games and recreation	120 min I/II band
	General training+endurance training	120 min I/II band	Strength training	90 min I/II band
Thurs.	Sports, games and recreation	120 min II/III band	Training on egometer	90 min I band
	Training on water	120 min I/II band	General training	120 min I/II band
Fri.	Training on water+running	120 min I/II band	General training	120 min I band
	Strength training for strength	90 min II/III band	Strength training	90 min I/II band
Sat.	Running	90 min I/II band	General training	120 min I band
	Swimming, sport games and recreation	120 min II/III band	General training	120 min I band
Sun.	Strength training	90 min II/III band	Strength training	90 min I band

Training began on monday
I band—blood lactate level <4 mmol/l
II band—blood lactate level 4–8 mmol/l
III band—blood lactate level >8 mmol/l

Table 3
Activity of acid phosphatase (AcP), arylsulphatase (ASA), cathepsin D (CTSD), α-1-antitrypsin (AAT) and creatine kinase (CK) and the level of cortisol in blood serum of kayaker woman after the training cycle with twice a day whole-body cryostimulation in comparison to the training without cryogenic temperature exposure.

	Before the start of training (control)	Training without cryostimulation		Training with cryostimulation	
		After 6th day	**After 10th day**	**After 6th day**	**After 10th day**
AcP (nkat)	10.1 ± 1.7	$18.2 \pm 6.1^{**}$	13.1 ± 2.3	10.9 ± 2.9^{aa}	6.9 ± 2.5
ASA (nkat)	25.4 ± 6.4	$46.5 \pm 14.6^{**}$	44.0 ± 13.4	26.2 ± 3.2^{aa}	35.8 ± 11.9
CTSD (nkat)	794.1 ± 96.5	1179.7 ± 229.1	$1721.1 \pm 534.2^{**}$	798.5 ± 154.1	$1915.5 \pm 541.0^{***ccc}$
AAT (mg/ml)	1.20 ± 0.57	$0.42 \pm 0.13^{**}$	$0.14 \pm 0.03^{**}$	1.21 ± 0.44^{aa}	1.02 ± 0.38^{b}
CK (IU/l)	72.0 ± 18.1	$231.7 \pm 70.0^{***}$	$212.8 \pm 66.7^{**}$	$237.0 \pm 67.8^{***}$	$196.9 \pm 55.1^{**}$
Cortisol (μg/dl)	21.8 ± 4.3	$30.6 \pm 4.8^{*}$	30.1 ± 4.6	24.7 ± 4.2	28.5 ± 6.6

statistically significant differences:

- versus control: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.
- versus training without cryostimulation after 6th day: $^{a}p<0.05$, $^{aa}p<0.01$, $^{aaa}p<0.001$.
- versus training without cryostimulation after 10th day: $^{b}p<0.05$, $^{bb}p<0.01$, $^{bbb}p<0.001$.
- versus training with cryostimulation after 6th day: $^{c}p<0.05$, $^{cc}p<0.01$, $^{ccc}p<0.001$.

(Table 3). After the 6th day of training cycle with and without WBC it was more than three-fold higher ($p<0.001$) than control. Although after 10th day of CK activity was a bit lower than after 6th day, it was still almost three-fold higher ($p<0.01$) than before the start of the study. Considering the level of cortisol, after the 6th day of training without cryostimulation it was about 40% higher ($p<0.05$) than before the start of the training (Table 3). After the 10th day it still remained higher, but the difference was statistically insignificant. During the training with use of cryogenic temperatures no statistically significant alterations of this stress hormone were found.

4. Discussion

In presented study the activity of certain lysosomal enzymes was elevated in blood serum of kayaker women after the training without application of whole-body cryostimulation. The increase in lysosomal enzymes activity as a result of intense exercise was previously reported by other authors. Bakońska-Pacoń et al. (2005) demonstrated that maximal exercise affects the activity of lysosomal enzymes, like arylsulphatase and beta-glucuronidase in all type of rat muscular fibres. The total activities of several lysosomal acid hydrolases were also found to be higher in the skeletal muscles of mice and rats after a single bout of prolonged running (Salminen, 1985). The authors point to the fact that the level of histological injuries of muscle fibres was correlated with the lysosomal enzymes response thus they postulate that these enzymes participate in pathogenesis of exercise myopathy. The higher activity of lysosomal enzymes after physical training as compared with the value before the exercise was also revealed in rowers and kayakers (Drewa et al., 2000). In present study, we revealed the increase in the activity of acid phosphatase and arylsulphatase after training without WBC, but not after the training with application of extremely low temperatures. The obtained results suggest that the exposure to cryogenic temperatures have an impact on the activity of lysosomal enzymes. The decreased activity in lysosomal hydrolases may concur to the reduced formation of muscles micro-injuries, which are probably due to the increase in ability to do long-lasting exercise of high intensity after the whole-body cryostimulation. Chwalbińska (2002) demonstrated that after a series of cryostimulation sessions in a group of professional sportsmen, after the stepped exercise test on the cyclo-ergometer, the evident increase of trigger power within a range of anaerobic threshold referring to the lactate level of 4 mmol/l was found.

Considering the activity of cathepsin D, the highest activity of this enzyme was noticed after the 10th day of training preceded with application of extreme cold. The differences in activity profile

of lysosomal enzymes studied in this paper may result from the variety of their cellular function. The increase in lysosomal enzymes may not be confined to a muscle wasting, but also related to the processes of differentiation and development occurring during the regeneration of muscle fibres. The main enzymes responsible for protein catabolism hence the degradation of membrane proteins associated with local trauma are lysosomal proteinases (Farges et al., 2002). Carmeli et al. (2007) found that high-intensity running results in an elevation of enzymes involved in matrix protein degradation: cathepsin D and matrix metalloproteinase 9. The proteolytic enzymes play an important role in the homeostasis of the extracellular matrix surrounding skeletal muscle, which in turn, provides structural support and protection, as well as it is important in maintaining of functional integrity of the fibres (Carmeli et al., 2005). Whole-body cryostimuation may have an influence of this homeostasis and high activity of cathepsin D in serum of kayaker women after the 10th day of training preceded by WBC may be a manifestation of intense regeneration processes within the muscle fibres. Restoration of plasma membrane activity after injury is essential for survival of cells and the main mechanism responsible for membrane repair is calcium-regulated exocytosis of lysosomes (Reddy et al., 2001). Ca^{2+}-dependent exocytosis is supposed to act by providing membrane patch or by relieving plasma membrane tension, which facilitates resealing (Idone et al., 2008). We suppose that the intracellular concentration of calcium may be affected by extremely low temperatures. Aslanidi et al. (1997) showed the cold-induced drastic increase in the basal concentration of cytoplasmic Ca^{2+} as a result of decrease in calcium removal from the cells with concurrent increase of calcium influx both from outside of the cells and from cellular organelles.

In investigated group of kayaker women, the intense training significantly reduced α_1-antitryspin activity. The inhibiting activity of AAT may be rendered by at least two mechanism based on the damage of its active centre either by reactive oxygen species (ROS) or proteinases, including matrix metalloproteinases (Zhang et al., 1993). Activation of some matrix metalloproteinases was found to be connected with various myopathic and inflammatory induced changes in skeletal muscle (Carmeli et al., 2005). Intense exercise is also known to induce the intensified generation of ROS (Woźniak et al., 2007) and so the oxidation of AAT is a possible mechanism of its inactivation in studied group of sportswomen during the training without cryostimulation. The lack of changes in α_1-antitryspin activity after the training cycle, during which the exercise was preceded by whole-body cryostimulation may provide the evidence of antioxidant thus anti-inflammatory properties of cryogenic temperatures application. The improvement of antioxidant capacity of organism exposed to intense exercise was previously reported at the same group of kayaker women (Mila-Kierzenkowska et al., 2009).

The synthesis and the certain level of α_1-antitryspin in monocytes is a serious agent that protects the tissues against the damage by proteolysis (Janciauskiene and Lindgren, 1999). The AAT production in monocytes, which are able to phagocyte is lower than in hepatocytes, nevertheless they play an important role in maintaining the proteases/antiproteases balance thus preserve the tissues damage in a place of inflammatory where the monocytes are gathered. α_1-antitryspin as an anti-inflammatory component participates in acute phase response and the increase in activity of this protein inhibitor after intense exercise have been demonstrated (Semple et al., 2006). These reports are in contrary to our own study as after an intense exercise we found a significant decrease of AAT activity. α_1-antitryspin seems to be one of those element of organisms response to exercise that depend on type of training, its intensity and duration, and even on training experience of competitors. Fallon et al. (2001) reported the statistically significant increase inf AAT activity after the moderate training and statistically insignificant decrease in its activity after heavy training at the same group of women soccer players. We suppose that WBC likely as moderate training may be a natural stimuli of organism, which does not include this protein in organism response. Nevertheless, the intense exercise itself may lead to induction of greater number of agents, including acute phase proteins with anti-inflammatory properties like α_1-antitryspin.

In presented study no significant differences in creatine kinase activity were revealed as comparing the training without and with application of whole-body cryostimulation. Sipaviciene et al. (2008) demonstrate that cooling of the lower limbs applied after exercise decreases the activity of creatine kinase and causes the faster recovery of muscle strength. The decrease in CK total activity after whole-body cryotherapy in athletes was also demonstrated by Banfi et al. (2008). However, at the same group of sportsmen other biochemical markers of muscle damage, like troponin I and C-reactive protein remained unchanged. The result obtained in presented study also revealed no statistically significant differences between training cycle with and without cryostimulation while considering the level of cortisol. After the sixth day of training without cryostimulation it was statistically significant higher than before the start of the study and it was the only one statistically significant difference. However, some decreasing tendency seems to occur during the training with exercise preceded by cryostimulation as compared to the training without application of extremely low temperatures.

The intense exercise is usually responsible for certain changes in the organism that may perturb cellular homeostasis thus lead to a loss of normal cell function. However, the recovery from the perturbations is possible upon the return of pre-exercise conditions. According to the hormetic theory cellular adaptations, which occur under different stressors may tend to decrease the exercise-induced perturbations and facilitate to maintain the normal cell function. Such a factor that positively affects the kayaker women's organism seems to be whole-body cryostimulation.

5. Conclusions

In studied group of kayaker women the common marker of muscle damage like creatine kinase remains unchanged and the stress hormone—cortisol shows only a decreasing tendency after the WBC. Nevertheless, we found significant changes in activity of lysosomal enzymes and activity of protease inhibitor, which are also accredited parameters of post-exercise changes in organism. Thus we propose that those proteins may be novel indicators of the effects of application of whole-body cryostimulation in sportsmen.

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